Molecular discrimination of sessile rectal adenomas from carcinomas for a better treatment choice: integration of chromosomal instability patterns and expression array analysis

Lips, E.H.

Citation

Lips, E. H. (2008, June 19). *Molecular discrimination of sessile rectal adenomas from carcinomas for a better treatment choice: integration of chromosomal instability patterns and expression array analysis*. Retrieved from https://hdl.handle.net/1887/12962

Version:	Corrected Publisher's Version
License:	Licence agreement concerning inclusion of doctoral thesis in the Institutional Repository of the University of Leiden
Downloaded from:	https://hdl.handle.net/1887/12962

Note: To cite this publication please use the final published version (if applicable).

Summary

This thesis describes the use of molecular analyses for the classification of rectal tumors. **Chapter 1** gives a general introduction for this thesis and describes the aim and research questions. Rectal cancer has an incidence of almost 300,000 new cases per year in Europe and the USA. The incidence of rectal adenomas is even higher. A recently introduced endoscopic microsurgical approach, TEM, exhibits reduced morbidity for the treatment of rectal adenomas. In spite of preoperative staging using ERUS and CT/MRI, carcinomas are frequently found after TEM treatment, indicating an increased risk for local recurrence and lymph node metastases. Following local recurrence after TEM, an additional total mesorectal excision (TME) is required, with extra morbidity or even mortality of these often elderly patients. Therefore, the identification of preoperative parameters that correctly identify any aggressive behavior of a tumor is crucial.

The aim of the research described in this thesis was to improve the classification of rectal tumors through molecular analyses.

A common characteristic of rectal cancer is chromosomal instability, characterized by genomic copy number variation and loss of heterozygosity (LOH). Recently introduced single nucleotide polymorphism (SNP) arrays can be used for the detection of copy number changes and LOH. Currently, most platforms require genomic DNA obtained from frozen tissue; however, for most cancer cases with long-term follow-up, only fragmented DNA from formalin-fixed, paraffin-embedded (FFPE) material is available. To profile multiple preoperative biopsies (i.e., FFPE material for standard histopathology), we developed protocols and algorithms for typing FFPE tissues using a new SNP array platform. **Chapter 2** describes the genotyping and LOH analysis on Illumina BeadArrays, and data from FFPE and frozen tumor samples were compared. Although these analyses used genotypes of the SNPs, the intensity values can be used to measure relative copy number. In **Chapter 3,** new software algorithms for copy number analysis using the BeadArray technology were developed and applied to a well-characterized set of colorectal tumors. In these studies, we showed that FFPE tissue can reliably be genotyped, and genome-wide LOH and copy number profiles can be determined. This enables high-throughput, genome-wide genotyping of archived FFPE tissue blocks not only for LOH and copy number analysis, but also for linkage studies and other applications. In the near future, the BeadArray technology on FFPE tissue can be applied in preoperative diagnostics for clinical decision making.

In **Chapter 4,** rectal tumors of various stages were analyzed for LOH and copy number changes using SNP arrays. Five specific genomic events (loss of 17p and 18q, and gain of 8q, 13q, and 20q) were identified that, when applied to a quantitative model, were able to discriminate rectal adenomas from rectal carcinomas. In a small set of adenomas containing a malignant focus, increased percentages of these “malignant” events were observed when compared to pure adenomas. In addition, the gain of 1q was related to

lymph node metastasis. These data show the potential of chromosomal aberration patterns for preoperative staging of rectal tumors.

For the correct preoperative staging of rectal tumors, especially large adenomas eligible for endoscopic TEM treatment, it is necessary to identify those adenomas already containing an invasive focus. These tumors usually consist of a large adenoma fraction and a small carcinoma focus, usually not obtained during the snap-freezing of the tumor material. The FFPE SNP array technology (from Chapters 2 and 3) enabled us to perform LOH and copy number analysis on both tumor fractions using FFPE material and to validate the model from Chapter 4 in a larger series. In **Chapter 5,** 36 paired FFPE rectal adenomas and carcinomas were analyzed for the specific LOH and copy number patterns associated with adenoma to carcinoma progression and were compared to pure adenomas. We observed early changes common to pure adenomas, progression events ("malignant" aberrations) in the transition from adenomas to carcinomas, and late events specific to carcinomas. Significantly, more "malignant" aberrations were observed in the adenoma fractions of carcinomas compared to pure adenomas. Immunohistochemistry for two target genes, i.e., *p53* (chromosome 17p) and *SMAD4* (chromosome 18q), showed aberrant staining in the adenoma fractions of carcinomas, in contrast to the pure adenomas. In individual cases, we were able to assess progression patterns by a systematic comparison of adenoma and carcinoma fractions in single lesions. Finally, tumor heterogeneity was assessed through analysis of three different tumor biopsies of each patient. These analyses showed a large degree of tumor heterogeneity, although a good correlation was obtained between the biopsy with the largest number of aberrations and its corresponding tumor fraction. Small core biopsies represent the tumor, but for a correct assessment of chromosomal changes it is necessary to study at least three different biopsies per patient. Moreover, the biopsy analysis showed that the FFPE SNP array technology analysis of small biopsies is feasible, indicating that it can reliably be used for preoperative biopsies as well.

In **Chapter 6,** gene expression array studies were performed on the same samples as the SNP array study from chapter 4; the chromosomal instability patterns were integrated with the gene expression array data. In general, we found a good correlation between the five specific "malignant" events and the expression of specific genes on the affected chromosomes. We identified specific genes where chromosomal aberrations directly lead to transcriptional changes. Validation studies were performed for *EFNA1* on chromosome 1q, *BOP1* on chromosome 8q, *SMAD2* on chromosome 18q, and *STMN3* on chromosome 20q, which were among the most significantly altered genes for the six chromosomes and were previously shown to be involved in (colorectal) cancer. Specific genes, identified by such integration methods, can potentially be used to classify early rectal cancer more exactly.

Finally, **Chapter 7** contains concluding remarks and implications for further research. In a follow-up study, we want to evaluate the sensitivity and specificity of the five "malignant" events, in combination with immunohistochemistry for SMAD4 and p53 on preoperative FFPE biopsies, for clinical decision-making. After extensive validation, we think that this method could offer a new tool for improved diagnosis of rectal tumors and could supplement current treatment criteria.