SUMMARY

Microsatellite instability (MSI) is the predominant type of genetic instability present in tumors of Lynch syndrome patients and in a subset (15-25%) of sporadic colorectal (CRC), endometrial (EC) and gastric tumors (GC). The underlying mechanism of MSI is a defect in the DNA mismatch repair (MMR) pathway. MSI is characterized by the accumulation of mutations in short, repetitive DNA sequences, also called microsatellites. This type of mutation can be found in both non-coding and coding microsatellites. This thesis presents a study on MSI in tumors from Lynch syndrome patients, with particular emphasis on colorectal and endometrial carcinomas and their sporadic counterparts.

In chapter 1 the general background to Lynch syndrome, microsatellite instability, and to the cancers traditionally associated with these MMR defects is presented. In chapter 2 we describe a study on how microsatellite instability evolves along the adenoma-carcinoma sequence of colorectal cancer. We found comparable MSI profiles, measured by the relative frequency of mono- and dinucleotide unstable markers, in sporadic colorectal adenomas and carcinomas. However, we found differences in MMR gene-truncating mutation carriers: colorectal adenomas showed instability almost exclusively in mononucleotide repeats whereas the frequency of dinucleotide marker instability was markedly increased in colorectal carcinomas. We concluded that MSI profiles differ between familial and sporadic cases, and that mononucleotide marker instability precedes dinucleotide marker instability during colorectal tumor development in Lynch syndrome patients. We therefore suggest that mononucleotide markers should be the preferred markers for identifying possible Lynch syndrome patients.

In chapter 3, we address whether the MSI profiles of colorectal and endometrial MSI-high (MSI-H) tumors differ. To answer this question we analyzed the frequency of the MSI, the type of mutation (deletions/insertions), and the size of the microsatellite mutation occurring at three mononucleotide repeat markers and at three dinucleotide markers in both CRC and EC. The frequency of mono- and dinucleotide instability found in both tissues was comparable, with mononucleotide and dinucleotide markers being affected at similar levels. We show that the type of mutation is a marker-dependent and not a tissue-dependent feature, since we
observed for both tissues almost exclusively deletions in mononucleotide markers, and both deletions and insertions in dinucleotide markers. The size of the deletions/insertions also differs between CRC and EC, with EC having shorter alterations than CRC. We concluded that there was no substantial difference between the MSI profiles of CRC and EC tumors. Furthermore, our data also showed that the same MSI tests could be used for both tumor types.

Chapter 4 describes our hunt for new target genes for MSI-H endometrial cancer. MSI is characterized by the accumulation of mutations in both non-coding and coding microsatellites, and genes containing microsatellites are frequent targets of mutations in MMR-deficient tumors. Particularly mutations in important regulatory genes – which we call target genes – are thought to be key players in the development of MSI-H related tumors. We set up an endometrium-specific strategy to find new target genes for MSI-H endometrial tumors. We screened genes that are expressed in normal endometrium tissue and that contain repetitive sequences. From a list of 2,338 genes expressed in the normal endometrium, 382 genes were found to contain 496 repeats and these genes were therefore sequenced. Mutations in these repeats were found in 44 genes, but whether all 44 genes really contribute to tumor development can be debated. A major criterion for selecting target genes that really contribute to tumors is the mutation frequency. Generally a cut-off of 15% is taken as revealing a real target gene. Genes mutated in lower frequencies are considered bystanders. When we applied this 15% cut-off, we found seven new, real, target genes. Subsequently we also analyzed 10 real EC target genes in colorectal and gastric MSI-H carcinomas. Our study confirmed that some target genes show tissue specificity, while others seem to play a more common role in MSI-H tumors, independently of the origin of the tissue.

The gene most frequently mutated in EC was NRIP1 (34%). This gene encodes a co-repressor protein of the estrogen-receptor (ER) pathway, one of the main pathways known to play a role in endometrial carcinogenesis. Surprisingly this gene was also highly mutated in CRC (24%). These results point towards an important role for the ER pathway in the development of MSI CRC tumors as well. Our data also suggest that genes of the ER pathway might be good candidates for target genes in MSI-H tumors, not only for estrogen-responsive tissues, such as the endometrium, but also for tissues of different origin such as the colon.
The findings described in chapter 4 led us to write a review in which we tried to clarify the mechanisms linking hormones to Lynch syndrome-associated tumors and, in particular, to discuss how hormones can play a role in MSI tumorigenesis.

In general our data has provided new insights into the process of MSI-H related tumor development: we propose that in the colorectal adenoma-carcinoma sequence of Lynch syndrome tumors mononucleotide instability precedes dinucleotide instability; we showed that there is no substantial difference of MSI profile between CRC and EC; and we found mutations that are likely to affect the estrogen-receptor pathway. Our data suggest that genes in the ER pathway are perfect candidate genes for mutation analysis in MSI-H, but possibly also in microsatellite-stable tumors. These findings might prove interesting in designing novel therapeutic treatments.