Improved Diagnostics of mismatch-repair defective cancer

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Background
Colorectal cancer affects 6000 Swedes annually and constitutes a prime example of how genetic changes accumulate during the progression from adenoma to carcinoma.

The presented studies evolve around defective mismatch-repair (MMR), which drives tumor development in a subset of colorectal cancer. MMR defects can be present either in the germline or be acquired as somatic changes during tumor development.

Germline MMR gene mutations lead to Lynch syndrome, which constitutes 2-4% of colorectal cancer. Lynch syndrome is characterized by young (mean 44) age at onset and high risk of synchronous/metachronous cancer. The highest risks apply to colorectal (50-80%) and endometrial (40-60%) cancer, but increased risks apply also to e.g. cancer of the ovary, urinary tract, stomach, and the small intestine. Lynch syndrome is caused by germline mutations in the mismatch repair genes MLH1, MSH2, MSH6 and PMS2. MSH2 mutations confer the highest risk of extracolonic tumors, whereas MSH6 mutations are linked to later age at onset and high risk for gynecologic tumors. Identification of families with hereditary colorectal cancer offers unique possibilities for cancer prevention in a high-risk population. Family history was initially used to identify individuals with Lynch syndrome, but has now been recognized as suboptimal since it fails to identify a substantial number of cases.

Also 15-20% of the sporadic colorectal cancers are characterized by MMR defects, which are caused by somatic hypermethylation of the MLH1 promoter. These tumors typically develop in the proximal colon and are overrepresented in female patients and at older age. A growing number of studies have shown a prognostic importance in this subset. The MMR defective tumors have a favorable prognosis, particularly in stages I-II, but may exhibit a poor response to 5-FU based chemotherapy, which implies that adjuvant therapy can be spared patients with stage II MMR defective colorectal cancer. The thesis work focuses on MMR defective tumors with links to refined diagnostics of hereditary cancer and improved prognostic tools.
Aims
The long-term aims of this PhD study include refined diagnostics and improved risk estimates for individuals affected by mismatch repair defects, in hereditary or sporadic form.

Study 1 - Do morphologic features allow recognition of mismatch-repair deficient colon cancers?
Study 2 – What causes heterogenous MMR expression in colorectal cancer?
Study 3 – Characterization of urological cancers in Lynch syndrome and estimates of the risk of tumor development.
Study 4 – Are MMR defective colon cancers after age 50 sporadic or may hereditary cases be obscured herein?

Studies
1. Efficient and reproducible identification of mismatch repair deficient colon cancer: validation of the MMR index and comparison with other predictive models
Patrick Joost, Pär-Ola Bendahl, Britta Halvarsson, Eva Rambech and Mef Nilbert

Background: The identification of mismatch-repair (MMR) defective colon cancer is clinically relevant for diagnostic, prognostic and potentially also for treatment predictive purposes. Preselection of tumors for MMR analysis can be obtained with predictive models, which need to demonstrate ease of application and favorable reproducibility.

Methods: We validated the MMR index for the identification of prognostically favorable MMR deficient colon cancers and compared performance to 5 other prediction models. In total, 474 colon cancers diagnosed ≥ age 50 were evaluated with correlation between clinicopathologic variables and immunohistochemical MMR protein expression.

Results: Female sex, age ≥60 years, proximal tumor location, expanding growth pattern, lack of dirty necrosis, mucinous differentiation and presence of tumor-infiltrating lymphocytes significantly correlated with MMR deficiency. Presence of at least 4 of the MMR index factors identified MMR deficient tumors with 93% sensitivity and 76% specificity and showed favorable reproducibility with a kappa value of 0.88. The MMR index also performed favorably when compared to 5 other predictive models.

Conclusions: The MMR index is easy to apply and efficiently identifies MMR defective colon cancers with high sensitivity and specificity. The model shows stable performance with low inter-observer variability and favorable performance when compared to other MMR predictive models.

2. Heterogenous mismatch-repair status in colorectal cancer
Patrick Joost, Nynke Veurink, Susanne Holck, Louise Klarskov, Anders Bojesen, Maria Harbo, Bo Baldetorp, Eva Rambech and Mef Nilbert
Diagnostic Pathology, 2014; 9:126.
**Background:** Immunohistochemical staining for mismatch repair proteins is efficient and widely used to identify mismatch repair defective tumors. The tumors typically show uniform and widespread loss of MMR protein staining. We identified and characterized colorectal cancers with alternative, heterogenous mismatch repair protein staining in order to delineate expression patterns and underlying mechanisms.

**Methods:** Heterogenous staining patterns that affected at least one of the mismatch repair proteins MLH1, PMS2, MSH2 and MSH6 were identified in 14 colorectal cancers. Based on alternative expression patterns macro-dissected and micro-dissected tumor areas were separately analyzed for microsatellite instability and MLH1 promoter methylation.

**Results:** Heterogenous retained/lost mismatch repair protein expression could be classified as intraglandular (within or in-between glandular formations), clonal (in whole glands or groups of glands) and compartmental (in larger tumor areas/compartments or in between different tumor blocks). These patterns coexisted in 9/14 tumors and in the majority of the tumors correlated with differences in microsatellite instability/MLH1 methylation status.

**Conclusions:** Heterogenous mismatch repair status can be demonstrated in colorectal cancer. Though rare, attention to this phenomenon is recommended since it corresponds to differences in mismatch repair status that are relevant for correct classification.

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**3. Urinary tract tumors in Lynch Syndrome**

*Patrick Joost, Christina Therklidsen, Steen Ladelund, Mev Dominguez-Valentin, Mats Jönsson, Eva Rambech and Mef Nilbert. 2014, in manuscript.*

**Background:** Lynch syndrome (LS) is caused by mutations in mismatch repair (MMR) genes. An increased frequency of urothelial cancer in the upper urinary tract has been described in mutation carrier, whereas the role of bladder cancer is uncertain.

**Methods:** We utilized the national Danish hereditary nonpolyposis colorectal cancer register to identify all urological cancers in the Lynch syndrome cohort and herein evaluated MMR status and estimated the cumulative life-time incidence for urothelial cancer.

**Preliminary results:** As expected, MSH2 mutations conferred a significant increased risk of cancer in the upper urinary tract compared to other MMR gene mutations (6.91%, p=0.0014). Additionally, we provide evidence that MSH2 mutation carriers have a significantly increased risk for bladder cancer of 4.40% (p=0.0081) compared to carriers of MLH1 and MSH6 mutations.

**Conclusions:** Lynch syndrome patients, in particular MSH2 mutation carriers, are at increased risk not only for cancer of the upper urinary tract and the bladder. The vast majority of the upper urinary tract tumors and half of the bladder cancers are MMR defective. Extended surveillance for cancer of the urinary tract could be considered in patients with MSH2 mutations.
4. Strategies for evaluation of MMR status in routine pathology.
Patrick Joost. 2015, work planned.

Background: Most pathologic laboratories suggest reflex analysis of MMR status in colorectal cancers diagnosed before age 50. This is motivated by a high number of sporadic MMR defective tumors and a presumed lower risk of Lynch syndrome in the patients aged 50 or older. We have, however, observed MMR defects suggestive of Lynch syndrome in up to 5% of colon cancer after age 50, which questions this approach.

Methods: We plan to evaluate MMR status (using immunostaining for MLH1, PSM2, MSH2, MSH6) in a series of 500 tumors from individuals >age 50 and 100 tumors from individual >age 40 related to effective diagnostic strategies.

Workplan: The project is planned for 2015.

Conclusion
Our studies have shown that:

- **Application of a MMR index may be used to identify MMR defective colon cancers with high sensitivity and specificity.**
- **Colorectal cancers may show heterogenous MMR expression, which also translates to heterogenous MSI status and this phenomenon should be reported accordingly.**
- **Individuals with Lynch syndrome are at increased risk of urinary tract cancer, which particularly related to MSH2 mutation carriers. Defective MMR is demonstrated in the vast majority of tumors of the upper urinary tract and in half of the bladder cancers in the cohort.**