Multiple-region deep sequencing of colorectal carcinoma in situ defines oncogenic transformation as a gradual and adaptive process

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Introduction Much is known about mutations in colorectal cancer (CRC), but little is known about evolutionary pathways connecting carcinomas to their ancestral adenomas. To investigate genetic alterations associated with the adenoma-carcinoma transition, we collected polyps isolated during routine colonoscopy which contained the first visible stages of colorectal neoplasia, so-called carcinomas in situ. These samples provided us with the earliest window of transition.

Material and methods We sequenced adenoma and carcinoma DNA separately using a CRC driver gene panel and compared single nucleotide variants (SNVs) and copy number alterations (CNAs). Using multi-regional sampling and a Bayesian Dirichlet clustering process we tracked sub-clonal progression pathways to CRC. In addition, we explored regional patterns of P53 using immunohistochemistry. Samples which revealed little discordance were additionally sequenced at the whole exome level.

Results and discussions Our results demonstrate that recurrent genetic alterations at the chromosomal level can precede APC initiating mutations. We find adenoma heterogeneity to be extensive, providing a ‘playground’ for the initiation of carcinomas. Indeed, heterogeneity in driver genes such as RAS and PIK3CA appears to be greater in adenoma compared to carcinomas. Additionally, our study shows P53 to be associated with, but not sufficient for, adenoma to carcinoma progression.

Conclusion Our work at the transition border between an adenoma and a carcinoma in the colon provides a further layer of complexity to the Vogelstein step-wise progression model of CRC.

Detection of high-risk prostate cancer biomarkers by RNA sequencing and qPCR method

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Introduction New prognostic biomarkers for prostate cancer have the potential to overcome the clinical challenge of therapy decision and overtreatment. Present diagnostic and prognostic tests are still limited in specificity resulting in a large number of false positives and unnecessary biopsies. Furthermore, they do not enable a proper stratification between men with a high risk for an aggressive disease course requiring comprehensive therapy scheme after surgery and men with a low risk of disease recurrence cured after prostatectomy or eligible for active surveillance. In particular, patients with Gleason score 6 and 7 tumours (low and mid stage) are difficult to stratify for the appropriate invasive BC (NMIBC) and muscle-invasive BC (MIBC). NMIBC commonly recurs and multiple tumours may be resected from the same patient over many years. This provides a unique opportunity to study the molecular events that occur during disease evolution. Some patients receive courses of intravesical chemotherapy, which may provide a potent selective advantage during disease evolution. To understand chemotherapy-induced or selected events in bladder cancer, tumours from patients who have undergone intravesical chemotherapy were analysed using next-generation sequencing and patterns of tumour evolution are being assessed through the analysis of DNA copy number and somatic mutations.

Material and methods Low-pass whole-genome sequencing was used to generate copy number (CN) profiles for tumour samples from 23 patients with recurrent NMIBC. Whole exome sequencing was performed on paired pre- and post-chemotherapy tumours for a subset of 8 patients. Samples are being analysed for mutational signatures and subclone composition.

Results and discussions In total, 67 tumours from 23 patients with NMIBC were analysed for CN alterations (CNAs). Loss of 9 p21.3, a region that includes the tumour suppressor genes CDKN2A and CDKN2B, was the most common event, seen in 64% of the tumours. Of these, two thirds were homozygous deletion events. Complete loss of a copy of chromosome 9 was the most common large-scale event observed in just under half of the tumours. Recurrent tumours from each patient tended to share CNAs and no CN alterations that specifically related to treatment were identified.

To examine tumour evolution, exome data will be assessed for mutational burden, mutational signatures and subclone composition. Comparisons between pre- and post- treatment tumours will identify changes potentially associated with chemotherapy treatment.

Conclusion Low-pass whole genome sequencing showed that CN changes tend to be shared amongst recurrent tumours from the same patient, suggestive of a monoclonal origin. Exome sequencing data will provide further information on tumour evolution and provide an insight into the influence of chemotherapy on these samples.

Abstracts
therapy or for active surveillance as conservative management approach.

**Material and methods** Therefore, we aimed to evaluate the potential of novel or known prognostic biomarkers in high-risk and low-risk prostate cancer tissues, and adjacent normal tissues by RNA sequencing and qPCR techniques. We also investigated tumour heterogeneity by including different foci from primary prostate cancer. A set of prognostic biomarker candidates was identified upon RNA sequencing of radical prostatectomy tumours of patients (n=25) with or without biochemical relapse (>5 year follow-up), as well as adjacent benign tissues. The candidate genes were retrospectively validated by qPCR method in the same and in an independent patient cohort (n=59). Expression variance of genes was investigated in different tumour foci of four primary tumours.

**Results and discussions** Overall, 16 prognostic biomarker candidates were selected from RNA sequencing data analysis according to differential expression between high-risk cancer, low-risk cancer and benign tissues. In total 10/16 candidates were technically sound upon qPCR of in the same cohort. We could clearly show that qPCR is a very robust and sensitive method to verify RNA sequencing data. Additionally, known tumour markers like AMACR and ERG showed expected signatures associated with the clinical phenotype and FISH-based gene fusion status. Data from different tumour loci indicated high expression variances across tumour sections. Independent validation of candidate genes could not confirm significant differential expression between the patient risk groups.

**Conclusion** Tumour heterogeneity might impede the detection and validation of diagnostic and prognostic biomarkers in primary prostate cancer tissues.

**PO-325 NOVEL RECURRENT HIGH-LEVEL AMPLIFICATIONS IN MICROSATELLITE STABLE COLORECTAL CANCER**

**Introduction** Colorectal cancer (CRC) is a molecularly diverse disease with few targeted treatment options. Focal and high-amplitude DNA copy number aberrations are potential tumour drivers, and their identification may contribute to improved therapeutic outcomes in small patient subgroups, as recently illustrated by targeting the over-expression of HER2 protein resulting from high-level ERBB2 amplification in KRAS wild-type metastatic CRC. However, few recurrent amplifications have been detected in CRC.

**Material and methods** We analysed focal high-level amplifications in 203 microsatellite stable (MSS) primary colorectal tumours using genome-wide high-resolution Affymetrix SNP6.0 arrays. The ASCAT algorithm was used to derive discrete allele specific copy number estimates.

**Results and discussions** The overall copy number profiles confirmed the frequent gains and losses previously described in MSS CRC studies. Extreme focal amplifications (defined as >15 copies and <50 genes in peak) were found in 77 regions in tumours from 22 unique patients, distributed across 16 different chromosome arms. Recurrent amplifications included ERBB2, which was amplified to 97, 27 and 22 additional copies in tumours from three individual patients (amplification frequency 1.5%). The transcription factor TOX3 (16q) was also recurrently amplified in 1.5% of the patients, while MYC (8q), CCND2 (12p) and a region on 10q22.3-q23.1, where ANXA11 was nominated as a likely target by GISTIC analysis, were recurrent in 1% of the patients. Regions with extreme and focal amplifications were also investigated for lower-amplitude aberrations (5–15 additional DNA copies), revealing a 3% amplification frequency of TOX3 in our cohort.

**Conclusion** We have identified several recurrent amplifications in cancer-critical genes in CRC MSS tumours, including the transcription factor TOX3, suggesting novel drug targets for preclinical studies.

**PO-326 IMPACT OF MIR-205–5 P AND MIR-425–5 P ON WNT AND AR SIGNALLING PATHWAYS IN CASTRATION RESISTANT PROSTATE CANCERTRANSITION**

**Introduction** Prostate cancer (PCa) is the second leading cause of cancer mortality in western countries. Prostate tumours initially respond well to androgen-deprivation therapy (ADT). Unfortunately, the majority of tumours evolve, after androgen deprivation, from a hormone-sensitive to a castration-resistant prostate cancer (CRPC). For that reason new agents, targeting the androgen receptor (AR) pathway (abiraterone, enzalutamide among others), have been approved in the last decade. Unfortunately, the emergence of resistance to these treatments is common and CRPC remains highly lethal. Therefore, new approaches and better knowledge of the molecular mechanisms leading to CRPC is still needed. Mechanisms related to CRPC transition include increased expression of AR and activating mutations in this receptor. As in other tumours, there are other signalling pathways that could interfere with AR activation such as Wnt signalling pathway, which has been suggested to play an important role in CRPC. On the other hand, emerging evidences indicate that certain miRNAs are involved in the appearance of treatment resistances in several diseases. The aim of this project was to study miRNA and mRNA expression profiles to identify deregulated miRNAs and genes involved in the Wnt signalling pathway in CRPC.