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Topoisomerase-1 and -2A gene copy numbers are elevated in mismatch repair-proficient colorectal cancers

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ABSTRACT

Introduction: Topoisomerase 1 (*TOP1*) and 2A (*TOP2A*) are potential predictive biomarkers for irinotecan and anthracycline treatment, respectively, in colorectal cancer (CRC), and we have recently reported a high frequency of gene gain of the *TOP1* and *TOP2A* genes in CRC. Furthermore, Mismatch Repair (MMR) subtypes of CRC have been associated with benefit from adjuvant chemotherapy of primary CRC. Given the involvement of the topoisomerase enzymes in DNA replication and repair, we raised the hypothesis that an association may exist between *TOP* gene copy numbers and MMR proficiency/deficiency in CRC.
Material and methods: Test cohort: FISH analysis with an in-house *TOP1*/CEN20 probe mix and a commercially available *TOP2A*/CEN17 (Dako, Glostrup, Denmark) probe mix was performed on archival formalin fixed paraffin embedded (FFPE) tissue samples from 18 patients with proficient MMR (pMMR) CRC and 18 patients with deficient MMR (dMMR) CRC. *TOP1* and *TOP2A* gene copy numbers and their ratios per nucleus were correlated with MMR status using the Mann–Whitney test. Validation cohort: FFPE samples from 154 patients with primary stage III CRC (originally included in the RANX05 study) were classified according to MMR status by immunohistochemical analysis using validated antibodies for

Abbreviations: *TOP1*, gene symbol for the gene encoding topoisomerase 1; *TOP2A*, gene symbol for the gene encoding topoisomerase 2 alpha; CEN17, symbol for the chromosomal region encoding centromere 17; CEN20, symbol for the chromosomal region encoding centromere 20; CRC, colorectal cancer; MMR, mismatch repair; pMMR, proficient mismatch repair; dMMR, deficient mismatch repair; FISH, fluorescence in situ hybridization; IHC, immunohistochemistry; FFPE, formalin-fixed paraffin-embedded; MSI, microsatellite instability; CIN, chromosomal instability; IDL, insertion/deletion loops; mCRC, metastatic colorectal cancer.

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MLH1, MLH2, MSH6 and PMS2, and information on *TOP1*, CEN20, *TOP2A* and CEN17 status was previously published for this cohort.

Results: The observed *TOP1* gene copy numbers in the 36 CRC test cohort were significantly greater ($p < 0.01$) in the pMMR subgroup (mean: 3.84, SD: 2.03) than in the dMMR subgroup (mean: 1.50, SD: 0.12). Similarly, the *TOP2A* copy numbers were significantly greater ($p < 0.01$) in the pMMR subgroup (mean: 1.99, SD: 0.52) than in the dMMR subgroup (mean: 1.52, SD: 0.10). These findings were confirmed in the validation cohort, where in the pMMR subgroup 51% had ≥2 extra *TOP1* copies per cell, while all tumors classified as dMMR had diploid *TOP1* status and mean *TOP2A* copy numbers were 2.30 (SD: 1.36) and 1.80 (SD: 0.31) ($p = 0.01$) in the pMMR subgroup vs. dMMR subgroup, respectively.

Discussion and conclusion: Our results show that *TOP1* and *TOP2A* gene copy numbers are increased in the pMMR subgroup. We propose that this preference may reflect a selective pressure to gain and/or maintain the gained extra copies of topoisomerase genes whose products are required to cope with high replication stress present in the pMMR tumors, thereby providing a survival advantage selectively in pMMR tumors. Future studies should test this concept and explore potential differences between pMMR and dMMR tumors in response to Top1 and Top2 inhibitors.

1. Introduction

An increasing understanding of the molecular biology of cancer and the development of new targeted drugs have created a platform for the development of predictive markers aiming at introduction of individualised therapies. The topoisomerase inhibitor irinotecan is included in treatment regimens for metastatic colorectal cancer (mCRC) but no predictive biomarkers for irinotecan efficacy have so far been substantially investigated and found useful in a clinical setting (National Comprehensive Cancer Network, 2014 (http://NCCN.org)).

The topoisomerase-1 (Top1) protein is the cellular target of campothecins and its derivatives (i.e. irinotecan) and thus represents a putative irinotecan predictive biomarker in CRC (Pommier et al., 2010). A large randomized clinical trial including material from 1313 patients with mCRC from the FOCUS trial showed a positive association between high Top1 protein expression investigated by immunohistochemistry (IHC) and benefit from irinotecan in combination with 5-fluorouracil as first-line treatment (Braun et al., 2008). An association between IHC Top1 protein expression and benefit from irinotecan-containing chemotherapy has also been reported in a non-randomized retrospective study with a cohort of 498 patients (Kostopoulos et al., 2009) but due to the design of this study no distinction between prognosis and prediction could be obtained (Boonsong et al., 2002), (Staley et al., 1999), (Paradiso et al., 2004), (Tsavaris et al., 2009). However, analyses of the CAIRO IHC study based on the same anti-Top1 antibody as the one used in the FOCUS study failed to confirm the results from the latter (Koopman et al., 2009), thereby leaving the notion of Top1 status as a potential biomarker for response to irinotecan uncertain.

Topoisomerase-2A (Top2A) is the cellular target of anthracyclines and two meta-analyses recently confirmed that breast cancer patients with increased *TOP2A* gene copy number had increased benefit from adjuvant anthracycline treatment (Di Leo et al., 2011), (Du et al., 2011). A number of small and non-randomized clinical studies have suggested a 10–20% objective response rate following anthracycline treatment of mCRC (Michaelson et al., 1982), (Wils, 1984) and we have now initiated a prospective phase II clinical trial in which metastatic CRC patients with *TOP2A* gene amplification in their cancer cells are offered treatment with the Top2 inhibitor epirubicin (EudraCTno. 2013-001648-79).

We have previously shown, in a cohort of 154 stage III CRC patients that both elevated *TOP1* and *TOP2A* gene copy numbers are frequent findings in metastatic CRC (Nygard et al., 2013); (Romer et al., 2013); (Smith et al., 2013). Furthermore, a newly published study including material from 78 mCRC patients also found frequent gains of the *TOP1* gene and an increased *TOP1*/CEN20 ratio and a borderline significant association between the *TOP1* gene copy numbers and objective response to second-line irinotecan-therapy (Nygard et al., 2014).

There are two main pathways described in CRC that are characterized by distinct patterns of genomic instability, the microsatellite instability (MSI) pathway comprising approximately 15% and the chromosomal instability (CIN) pathway comprising approximately 85% of sporadic CRC (Grady and Carethers, 2008). Defects in the mismatch repair (MMR) system are considered the main cause of sporadic CRC with microsatellite instability as well as the inherited Lynch syndrome. In the Lynch syndrome the MMR defect reflects germline mutations of the genes encoding MMR proteins and in sporadic CRC defective MMR is most often due to MLH1 promoter hypermethylation (Geiersbach and Samowitz, 2011). The MMR system is a post-transcriptional proofreading system that corrects base–base mismatches and insertion/deletion loops (IDL). Cells with deficient MMR (dMMR) fail to correct these errors during DNA replication resulting in base substitutions and frameshift mutations. The repetitive base sequences in DNA, the microsatellites, which are present in both gene coding regions and gene regulatory regions, are especially prone to mutations, and the longer the microsatellite the greater frequency of mutations. This