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Global DNA methylation is altered by neoadjuvant chemoradiotherapy in rectal cancer and may predict response to treatment – A pilot study ☆

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Abstract

Aim: In rectal cancer, not all tumours display a response to neoadjuvant treatment. An accurate predictor of response does not exist to guide patient-specific treatment. DNA methylation is a distinctive molecular pathway in colorectal carcinogenesis. Whether DNA methylation is altered by neoadjuvant treatment and a potential response predictor is unknown. We aimed to determine whether DNA methylation is altered by neoadjuvant chemoradiotherapy (CRT) and to determine its role in predicting response to treatment.

Patients and methods: Fifty-three ($n = 53$) patients with locally advanced rectal cancers treated with neoadjuvant CRT followed by surgery were identified from the pathology databases of 2 tertiary referral centres over a 4-year period. Immunohistochemical staining of treatment specimens was carried out using the 5-Methylcytidine (Eurogentec, Seraing, Belgium) antibody. Quantitative analysis of staining was performed using an automated image analysis platform. The modified tumour regression grading system was used to assess tumour response to neoadjuvant therapy.

Results: Seven (13%) patients showed complete pathological response while 46 (87%) patients were partial responders to neoadjuvant treatment. In 38 (72%) patients, significant reduction in methylation was observed in post-treatment resection specimens compared to pre-treatment specimens (171.5 vs 152.7, $p = 0.01$); in 15 (28%) patients, methylation was increased. Pre-treatment methylation correlated significantly with tumour regression ($p < 0.001$), T-stage ($p = 0.005$), and was able to predict complete and partial pathological responders ($p = 0.01$).

Conclusion: Neoadjuvant CRT appears to alter the rectal cancer epigenome. The significant correlation between pre-treatment DNA methylation with tumour response suggests a potential role for methylation as a biomarker of response.

Keywords: Rectal cancer; DNA methylation; Neoadjuvant treatment

Introduction

Local recurrence is a crucial determinant of treatment success and prognosis in rectal cancer. Neoadjuvant chemoradiotherapy (CRT) followed by high quality surgery using total mesorectal excision (TME) significantly reduces local recurrence rates.[1,2] The benefit of this is clearly illustrated by the Dutch rectal cancer trial demonstrating a reduction in local recurrence rates from 8.4% to 2.4% at 2 years compared to TME alone.[3] In addition, neoadjuvant treatment has been shown to downstage rectal tumours, improve their resectability and may improve patient survival.[4,5]

Patients with tumours showing a pathological response to neoadjuvant treatment of any degree, benefit from improved local recurrence rates and better survival

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compared to non-responders.[6,7] The clinical significance of downstaging is highlighted by the 10–20% of patients who show a complete pathological response (CPR) to neoadjuvant CRT. These patients are characterised by an absence of residual tumour cells in the post-treatment resected specimens, have an excellent prognosis of >95% 5-year survival.[8–10]

However, not all patients benefit from neoadjuvant treatment. Partial responders show a spectrum of residual disease ranging from microscopic foci of adenocarcinoma to abundant macroscopic tumour cells quantified by a 3 or 5 point grading scale.[8] The variability of an individual patient's tumour responsiveness to CRT poses a significant clinical problem. Treatment is associated with increased cost and peri-operative morbidity including fistulae formation and perineal wound infections.[11] At present, all suitable patients receive neoadjuvant CRT but a proportion (10–20%) of patients have no clinical benefit and are subjected to the potential morbidity of therapy and delay in definitive surgery. Recent research has focused on identifying molecular biomarkers in pre-treatment rectal cancer biopsies to predict response and prognosis. Such a marker of response could potentially allow targeted patient-specific treatment by anticipating those unlikely to benefit from CRT and directing them to primary surgery. Many studies have examined different potential markers such as p21 and thymidylate synthase that play a role in the colorectal neoplastic pathway but results have been disappointing.[12–14]

The pathogenesis of colorectal cancer is well established. The adenoma-carcinoma sequence, characterized by a step-wise accumulation of genetic changes is responsible for the development of sporadic and familial adenomatous polyposis (FAP) cancers.[15] These molecular events occur in parallel to distinctive histo-pathological stages of polyp progression to eventual cancer. The novel molecular pathway involving epigenetic mechanisms is recognized as an additional, distinctive pathway to colorectal carcinogenesis. Unlike the traditional chromosomal and microsatellite instability pathways, which consist of direct mutational changes in key regulatory genes adenomatous polyposis coli (APC), K-Ras, p53 and human MutL Homolog 1 (hMLH1), epigenetic modifications involve regulatory changes that do not alter the DNA sequence. DNA methylation is the most widely understood epigenetic marker now shown to be responsible for a large variety of tumours including colorectal cancers. This process involves methylation (addition of CH_3-methyl groups) of promoter cytosine bases at the 5' position to form 5-methylcytosine (5-MC). Regulatory genes in neoplastic transformation are "silenced" by methylation, leading to suppression of individual gene expression. In colorectal cancer, both hyper and hypomethylation have been observed across different stages of progression.[16,17] The aim of this study was to evaluate the effect of neoadjuvant CRT on the rectal cancer epigenome and the potential of DNA methylation in predicting response to neoadjuvant treatment.

Patients and methods

Patients

The pathology database systems of two tertiary referral centres were searched for patients diagnosed with locally advanced rectal tumours treated with neoadjuvant CRT. Fifty-three ($n = 53$) patients were identified within a 4-year period of 2004–2008 and formed the study cohort. Pathological data of each rectal cancer specimen was collected by manually reviewing each patient's histology report from their primary surgery, and confirmation by slide review by a consultant pathologist. Each patient's initial tumour was at least T3 stage on pre-operative MRI examination. All patients had received the long-course CRT regimen (50.4 Gy in total; 1.8 Gy/day × 5 weeks, concomitant 5-fluorouracil continuous 120-h infusion at a dose of 1000 mg/m^2/day for 5 days during week 1 and 5) followed by surgery in the form of TME with either anterior resection or abdomino-perineal resection 4–6 weeks after completion of radiation. Ethical approval was granted by the local ethics committee.

Tissue processing and immunohistochemistry

Formalin-fixed paraffin-embedded tissue blocks of paired pre-treatment biopsies (PTB) and post-treatment resected specimens were retrieved from the pathology tissue banks of the two hospitals. Tissue blocks were cut into sections of 4 μm using a microtome and mounted on poly-lysine coated slides (Lahn-Chemical Co.). Consecutive sections were cut and stained with Haematoxylin and Eosin (H&E) and the commercially available murine 5-Methylcytidine antibody (Eurogentec, Seraing, Belgium) for DNA methylation detection. This monoclonal antibody preferentially binds to the modified (methylated) guanine bases and distinguishes them from the normal unmethylated guanine bases.

Immunostaining was performed using an automated immunostainer (Leica Microsystems, Bondmax, IL, USA). Firstly, deparaffinization was carried out using Bond™ Dewax solution (Leica Microsystems, IL, USA). This was followed by antigen retrieval using Bond™ Epitope Retrieval solution (Leica Microsystems®, IL, USA) and incubation for 20 min. Automated immunostaining was then commenced. Specimens of normal large bowel mucosa were used as a positive control. The primary antibody was omitted and staining repeated as a negative control.

Image analysis

Quantification and analysis of methylation staining was performed using the Aperio™ (Vista, CA, USA) automated