European Journal of Cancer (2013) 49, 254–263

Available at www.sciencedirect.com

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journal homepage: www.ejcancer.info

Mucosal immune environment in colonic carcinogenesis: CD80 expression is associated to oxidative DNA damage and TLR4–NFκB signalling

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In memory of Prof Attilio Cecchetto

Available online 14 June 2012

KEYWORDS
Colorectal cancer
Ulcerative colitis
Colonic cancerogenesis
Immunosurveillance
CD80
TLR4
NF-κB
8-OHdG

Abstract ***Background:*** CD80 has been thought to play an active role in immunosurveillance as it has been found to be up-regulated in ulcerative colitis (UC) patients with dysplasia. The aim of the present study was to analyse early events in UC-related and non-inflammatory carcinogenesis with reference to CD80 expression to clarify what stimuli are involved in its up-regulation in these patients.

Patients and methods: Sixty-two patients affected with UC, UC with dysplasia, UC and cancer, colonic adenoma, or colonic cancer and 11 healthy subjects were enroled in our study. Tissue samples were taken from surgical specimens during colonic resection or during colonoscopy. Mucosal mRNA expression of Toll-like receptor-4 (TLR4) and nuclear factor-kappaB (NF-κB) was quantified with Real Time RT-PCR. TLR4, β-catenin and p53 expressions were analysed by immunohistochemistry. Mucosal levels of activated NF-κB were measured with immunometric assays while 8-Hydroxydeoxyguanosine (8-OHdG) levels were quantified by high-performance liquid chromatography with electrochemical detection (HPLC-ED). Non-parametric tests were used for statistical analysis.

Results: 8-OHdG mucosal levels were higher in the patients with UC + dysplasia with respect to those in the patients with UC only ($p = 0.03$). CD80 mRNA mucosal levels were directly correlated with 8-OHdG mucosal levels ($\tau = 0.26$, $p = 0.04$), TLR4 protein expression

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0959-8049/$ - see front matter
http://dx.doi.org/10.1016/j.ejca.2012.05.015

($\tau = 0.45$, $p < 0.01$) and NF-κB mRNA expression and activity ($\tau = 0.24$, $p = 0.02$; $\tau = 0.34$, $p = 0.02$, respectively). CD80 protein expression, instead, was directly correlated with 8-OHdG mucosal levels ($\tau = 0.19$, $p = 0.05$) and inversely correlated with TLR4 mRNA expression ($\tau = -0.25$, $p = 0.03$).

Conclusion: Oxidative DNA damage peaked in UC-related dysplasia and was found to be directly correlated to CD80 expression. The direct correlation between TLR4 protein expression and CD80 mRNA and the indirect correlation between CD80 protein and TLR4 mRNA expressions give substance to the hypothesis that they play a role in immunosurveillance. No significant correlations between CD80 expression and p53 and β-catenin accumulation during oncogenesis were, instead, observed.

1. Background

Ulcerative colitis (UC) is one of the two main forms of chronic inflammatory disease of the gastrointestinal tract. Patients affected by UC have an approximate 8% cumulative risk of developing colorectal cancer within 20 years of the initial diagnosis and up to an 18% risk within 30 years.[1] Low grade dysplasia has been diagnosed in about 25% of UC patients during a 10 year follow-up study, but it has been hypothesised that dysplasia develops in all if the follow-up is long enough.[2] The discrepancy between the dysplasia rate and the actual cancer incidence suggests that mechanisms may be at play to prevent cancer progression. Cancer immunoediting could be a process leading to neoplastic cell destruction (immunosurveillance) mediated by T-cells, macrophages, and natural killer cells.[3] Activation of T-cells requires engagement of T-cell receptors with antigen-specific, major histocompatibility complex restricted receptors in the presence of adequate co-stimulation.[4] Co-stimulatory signals are provided by CD80 or CD86 on the surface of antigen-presenting cells interacting with co-receptors expressed by T-cells.[5] Indeed, oncogenic insults can induce CD80 expression that modulates the anti-tumour immune response.[6] A significant, specific overexpression of CD80 in the colonic mucosa of UC patients with dysplasia and a down-regulation at later stages of carcinogenesis were reported by us in a precedent study.[7,8] The precise molecular mechanisms involved in CD80 up-regulation during UC-related colonic carcinogenesis are, nevertheless, unclear.

The sequential pathway of carcinogenesis in UC is influenced by the production of reactive oxygen intermediates (ROIs) due to chronic inflammation.[9,10] Increased ROIs may cause DNA damage leading to strand breaks and adduct formation.[11] In fact, ROIs may interact with genomic DNA, producing several base modifications, such as 8-OHdG, formed by the reaction of the hydroxyl radical at the C-8 position of the de-oxyguanosine DNA base[12] or such as etheno-DNA adducts induced by DNA-reactive aldehydes.[13] DNA damage can lead to oncogene activation and suppressor gene inactivation.[14] Indeed, significantly higher mucosal 8-OHdG concentrations have been reported in UC patients with dysplasia by our group in a previous report.[15]

The loss of p53 function is one of the most frequent alterations in colorectal cancer[16] and nearly 80% of p53 mutations in UC-associated lesions are transition ones that can be caused by oxidative DNA damage.[17] The p53 signalling pathway regulates cellular senescence and is a critical mechanism for tumour suppression *in vivo*.[18] p53 mutations seem to occur early in neoplastic transformation in UC patients and are frequently noted in those who have had the disease for more than 10 years.[19] Mutations in the Adenomatous Polyposis Coli (APC) gene occur early in colorectal carcinogenesis leading to β-catenin accumulation in the nucleus where it acts as a transcription factor for proliferation genes. Less frequent than in sporadic carcinomas, from 0% to 50% of UC-related tumours show APC mutations,[20,21] while β-catenin mutations are equally infrequent in both tumour groups.[22] Hypothetically, p53 or β-catenin accumulation might trigger CD80 up-regulation in mutated cells.

CD80 expression can be regulated throughout the TLR4 and NF-κB signalling pathways. Thus, lipopolysaccharide (LPS) binding to TLR4 significantly up-regulates major histocompatibility complex (MHC), CD40, CD80 and CD86.[23] The immunogenicity of dying tumour cells after chemotherapy or radiotherapy depends on the release of the TLR4 ligand high-mobility-group box 1 (HMGB1).[24] Indeed, LPS stimulation can elicit different responses in a variety of cell populations in terms of pro-inflammatory cytokine secretion and expression of the co-stimulatory molecules CD80 and CD86.[25] The aim of this work was to analyse the interaction between early events in colonic UC-related and non-inflammatory carcinogenesis and CD80 expression to clarify what stimuli induce its up-regulation in these patients.

2. Patients and methods

As described elsewhere,[8] the study population, made up of 36 patients belonging to a UC-related carcinogenesis cohort, were diagnosed on the basis of clinical, laboratory, endoscopic and histological findings as: UC, UC with dysplasia, or UC with cancer. There were also