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Influence of myeloperoxidase on colon tumor occurrence in inflamed versus non-inflamed colons of $Apc^{Min/+}$ mice ☆

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ABSTRACT

Control of colorectal cancer needs to be tailored to its etiology. Tumor promotion mechanisms in colitis-associated colon cancer differ somewhat from the mechanisms involved in hereditary and sporadic colorectal cancer. Unlike sporadic or inherited tumors, some experimental models show that colitis-associated colon tumors do not require cyclooxygenase (COX) expression for progression, and non-steroidal anti-inflammatory drugs (NSAIDs) which prevent sporadic or inherited colon cancer do not prevent colitis-associated colon cancer. We report that myeloperoxidase (MPO), an ancestor of the COX isoenzymes, is a determinant of colitis-associated colon tumors in $Apc^{Min/+}$ mice. During experimentally induced colitis, inhibition of MPO by resorcinol dampened colon tumor development. Conversely, in the bowels of $Apc^{Min/+}$ mice without colitis, resorcinol administration or 'knockout' of MPO gene coincided with a slight, but discernible increase in colon tumor incidence. Acrolein, a by-product of MPO catalysis, formed a covalent adduct with the phosphatase tensin homolog (PTEN) tumor suppressor and enhanced the activity of the Akt kinase proto-oncogene in vitro and in vivo. Thus, MPO may be an important determinant of diet and inflammation on colon cancer risk via its effect on endogenous exposure to oxidants and acrolein. We propose a hypothetical model to explain an apparent dichotomy between colon tumor occurrence and MPO inhibition in inflamed versus non-inflamed colons.

1. Introduction

Chronic ulcerative colitis is an independent risk factor for colorectal cancer [1,2]. Consistent with epidemiological observations in humans, experimentally-induced colitis increases colon tumor progression in the $Apc^{Min/+}$ mouse, a model of intestinal cancer [3–7]. While the molecular pathways of inherited, sporadic and colitis-associated colon tumor progression overlap, they are not identical [8,9]. Tumor formation in some mouse models of colitis-associated colon cancer does not require cyclooxygenase (COX) expression [10]. In a study using COX-1 and COX-2 'knockout' mice, investigators concluded that the mechanism of colorectal tumor promotion in colitis-associated cancer differs from the mechanism of tumor promotion for hereditary and sporadic colorectal cancer [10]. However, the exact role of prostaglandins in colitis associated colorectal cancer models varies with the model [11]. Additionally, the pharmacological rationale for colon cancer prevention with non-steroidal anti-inflammatory drugs (NSAIDs) does not apply to colitis-associated tumors [12,13]. In fact, NSAIDs aggravate inflammation and malignant progression in rodent models of colitis-associated tumors [14–16], albeit with some exceptions [17]. One NSAID, 5-aminosalicylic acid, can maintain remission of ulcerative colitis, which ought to prevent colitis-associated colon cancer. However, that hypothesis is unproven despite many attempts at validation [12,18].

Myeloperoxidase (MPO), an ancestor of COX enzymes [19], helps gut associated lymphoid tissue defend against harmful enteric microbes, while tolerating harmless commensal bacteria and dietary antigens. Because MPO activity correlates with the severity of experimentally induced colitis [20] and its expression is an

Abbreviations: Apc, adenomatous polyposis coli; BME, β-mercaptoethanol; COX, cyclooxygenase; DTT, dithiothreitol; DSS, dextran sodium sulfate; ECL, enhanced chemiluminescence; EPO, eosinophil peroxidase; FBS, fetal bovine serum; HRP, horse radish peroxidase; MBTH, 3-methyl-2-benzothiazolinone hydrazone hydrochloride; MEM, modified Eagle's medium; MPO, myeloperoxidase; NSAIDs, non-steroidal anti-inflammatory drugs; PBS, phosphate buffered saline; PI3, phosphatidylinositol; PTEN, phosphatase and tensin homolog on chromosome 10; PVDF, polyvinylidene difluoride; WT, wild type

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indicator of colon cancer risk [21] we investigated its influence on colon tumor development in $Apc^{Min/+}$ mice. We report that elevated MPO activity in the inflamed bowels of $Apc^{Min/+}$ mice correlated with greater colon tumor occurrence; inhibition of MPO activity in inflamed colons of $Apc^{Min/+}$ mice partly suppressed colon tumor occurrence. Conversely, tumors were absent or rare in non-inflamed colons with low, basal MPO activity in $Apc^{Min/+}$ mice. Unexpectedly, either pharmacological or genetic suppression of basal MPO activity correlated with a small, but discernible rise in colon tumors in $Apc^{Min/+}$ without colitis. Thus, the relationship between MPO activity and colon tumor occurrence in $Apc^{Min/+}$ mice varies with the status of inflammation in the gut. Our mechanistic experiments found that a carcinogenic by-product of MPO catalysis, acrolein, formed a protein adduct with phosphatase tensin homolog (PTEN) in colonocytes isolated from the inflamed bowel of $Apc^{Min/+}$ mice. Modification of the PTEN tumor suppressor coincided with activation of the Akt kinase proto-oncogene, which favors cell growth and survival. Since acrolein can originate endogenously from MPO mediated oxidation of threonine or serine [22] this mechanism may contribute to complex effects of diet on inflammation and colon cancer risk.

2. Materials and methods

2.1. Reagents

The following were used: resorcinol, acrolein, L-threonine, hydrogen peroxide (H_2O_2) and 3-methyl-2-benzothiazolinone hydrazone hydrochloride monohydrate (MBTH) (Sigma Aldrich, St.

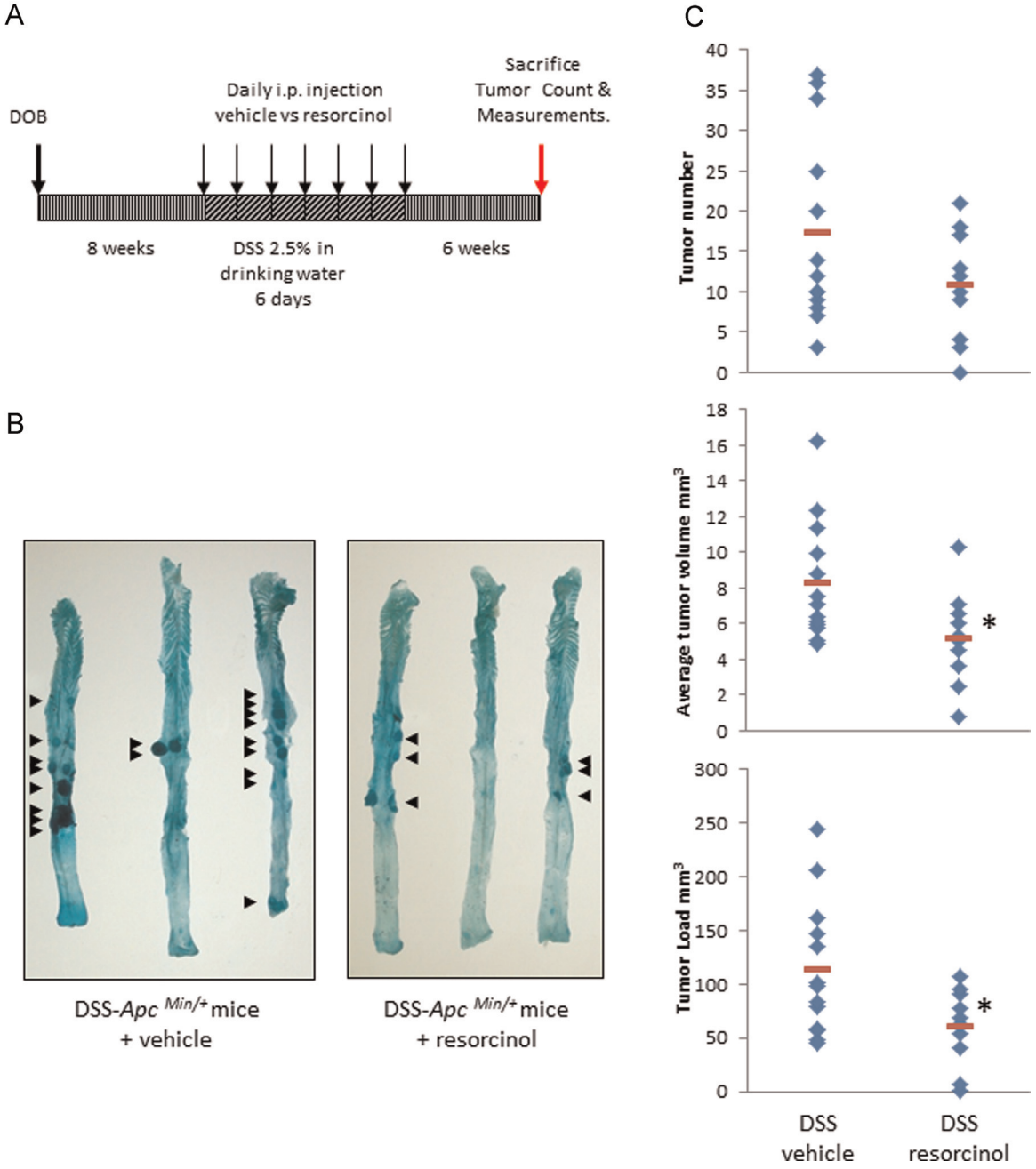

Fig. 1. Myeloperoxidase and colitis-associated colon tumors in $Apc^{Min/+}$ Mice. Panel A: protocol for induction of colitis with dextran sodium sulfate (DSS) and modulation of MPO activity with resorcinol in $Apc^{Min/+}$ mice. Panel B: colitis-associated tumors (arrows) in representative colons from DSS-$Apc^{Min/+}$ mice treated with vehicle or resorcinol. Panel C: graphs of colon tumor numbers, volumes and loads in colons from DSS-$Apc^{Min/+}$ mice treated with vehicle ($n=13$) or resorcinol ($n=10$).