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Original article

Upregulation of MAPK/Erk and PI3K/Akt pathways in ulcerative colitis-associated colon cancer

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ABSTRACT

An extracellular signal like a cytokine or chemokine, secreted in the inflammatory microenvironment can activate the mitogen activated protein kinase (MAPK) pathway by binding to a cytokine receptor tyrosine kinase, which further activates tyrosine kinases such as Janus Kinase-3 (Jak-3). This signal is transferred from Jak-3 to the DNA in the nucleus of the cell by a chain of kinases, ultimately activating extracellular receptor kinase (Erk/MAPK). The latter phosphorylates c-myc, an oncogene, which alters the levels and activities of many transcription factors leading to cell survival, proliferation and invasion. The oncogenic PI3K pathway plays a similar role by activating c-myc, leading to cell survival and proliferation. The present study explores the role of ulcerative colitis in colon cancer by investigating the activities of tyrosine kinase activated MAPK pathway and various components of the PI3K pathway including PI3K, PTEN, PDK1, GSK3β, Akt, mTOR, Wnt and β-catenin. This was done by western blot and fluorescent immunohistochemical analysis of the above-mentioned proteins. Also, the morphological and histological investigation of the colonic samples from various animal groups revealed significant alterations as compared to the control in both inflammatory as well as carcinogenic conditions. These effects were reduced to a large extent by the co-administration of celecoxib, a second-generation non-steroidal anti-inflammatory drug (NSAID).

1. Introduction

Chronic inflammation develops through the action of various inflammatory mediators like cytokines, chemokines and growth factors, which eradicate anti-tumor immunity and facilitate tumor progression [1]. TNF-α, a cytokine, has been found to be involved in all the stages of carcinogenesis such as cellular transformation, promotion, survival, proliferation, angiogenesis and metastasis [2]. Our previous studies have shown that the presence of such cytokines in the inflammatory milieu might lead to the transformation of cytoplasmic inactive transcription factor, nuclear factor κB (NF-κB) to its active nuclear form, thereby leading to tumorigenesis [2]. Presently, we attempt to look further into the aspects of inflammation associated colon cancer by studying the tyrosine kinases activated MAPK/Erk pathway. The latter is well known to facilitate the upregulation of several oncogenic agents including the PI3K pathway [3].

Tumor-related inflammation is an important hallmark of cancer. It contributes to almost every aspect of tumor development [4]. Several epidemiological evidences show that chronic inflammation of the colon (ulcerative colitis) may lead to colon carcinoma [5]. The microenvironment, in case of ulcerative colitis, secretes a large number of proinflammatory cytokines like TNF-α, IL-4 and IL-1β [6]. These cytokines may act as extracellular growth factors to activate a chain of kinases, beginning with a tyrosine kinase, Jak-3 [7]. This Jak-3 further activates a series of kinases, ultimately activating Erk.

Erk is responsible for cell survival, proliferation and invasion by further interacting with the oncogenic Phosphatidylinositol-3 kinase (PI3K) pathway [8]. Both the RAS-Erk and PI3K/Akt pathways are found to be activated in tumorigenesis. PI3K is a lipid kinase and generates phosphoinositol-3 phosphate (PIP3) while this reaction is reversed by phosphatase and tensin homolog (PTEN), the negative regulator of this oncogenic PI3K pathway [9]. PIP3 is a second messenger for the translocation of Akt to the

Abbreviations: DSS, dextran sulfate sodium; DMH, 1,2 dimethyl hydrazine; Erk, extracellular receptor kinase; GSK3β, glycogen synthase kinase 3β; Jak-3, Janus kinase-3; MAPK, mitogen activated protein kinase; MPL, multiple plaque lesions; NSAID, non-steroidal anti-inflammatory drug; PI3K, phosphatidylinositol-3 kinase; PTEN, phosphatase and tensin homolog.

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plasma membrane where it is phosphorylated and activated by PDK1. Activation of Akt plays a pivotal role in the fundamental cellular functions such as cell proliferation and survival by phosphorylating its downstream substrates like Glycogen synthase kinase 3β (GSK3β) and thereby inactivating it [10]. GSK3β in its active form doesn't allow the activation of β-catenin [11]. But when inactivated, β-catenin complex is degraded and β-catenin is phosphorylated, it moves to the nucleus and induces the transcription of several cell survival and proliferation related proteins [12]. Upon the inactivation of GSK3β, another oncogenic pathway led by Wnt activates β-catenin [13].

The present study incorporated the use of an inflammation inducing agent, dextran sulfate sodium (DSS) and a carcinogenic agent 1,2 dimethyl hydrazine (DMH) in Balb/c mice, individually as well as in combination, to develop animal model for ulcerative colitis, colon carcinoma as well as colitis-associated colon cancer, respectively. Also, the chemoprevention of these diseases was studied by the administration of a second-generation NSAID, celecoxib. Numerous epidemiological studies have reported that the long-term use of NSAIDs is associated with a significant decrease in cancer incidence and delayed progression of the malignant disease [14]. The expression of Cox-2 and prostaglandins has been associated with various types of cancer and is directly proportional to their tissue aggressiveness including metastasis [15]. NSAIDs act primarily by the inhibition of the Cox enzyme [16]. But how these NSAIDs act in case of ulcerative colitis and prevention of colitis-associated colon cancer is still not known. We presently make an attempt at revealing the mechanism underlying colitis-associated colon cancer treatment with NSAIDs.

2. Materials and methods

2.1. Animal husbandry

Balb/c mice were procured from the Central Animal House, Panjab University, Chandigarh. Animals were maintained as per the principles and guidelines of the Ethics Committee on the use of experimental animals of Panjab University and with approved protocol. They were housed in polypropylene cages with a wire mesh top and a regularly changed husk bed with a maximum of 6–8 animals in each cage. The animal rooms were maintained at ambient temperature and provided with a room cooler or heater during the summer or winter months, respectively. The animals received food (rodent chow) and water ad libitum and were exposed to 12 h day/night photoperiod. They were acclimatized for 1 week and then assorted.

2.2. Experimental design

The present study consisted of eight groups:

- control, which received vehicle for NSAIDs, (0.5% (w/v) carboxymethyl cellulose per oral daily), vehicle for DSS (distilled water during three weeks of DSS administration in group 2) and DMH (subcutaneous injection of 1 mM EDTA-saline);
- DSS, the animals received three cycles of 3% DSS in distilled water, each for seven days followed by 14 days of tap water, to establish a model of ulcerative colitis [17];
- DMH, 18 weekly s.c. injections at 30 mg/kg to develop a model for colon carcinogenesis, as established earlier in our laboratory [18];
- celecoxib, 6 mg/kg as a chemopreventive agent;
- DMH + DSS;
- DSS + Cel;
- DMH + Cel;
- DMH + DSS + Cel in the above-mentioned respective doses.

This has been summarized in Fig. 1, where blue boxes represent DSS treatment, black arrow represents a single s.c. injection of DMH and green colored boxes show celecoxib administration p.o. daily (for interpretation of the references to color in this figure legend, the reader is referred to the web version of this article).

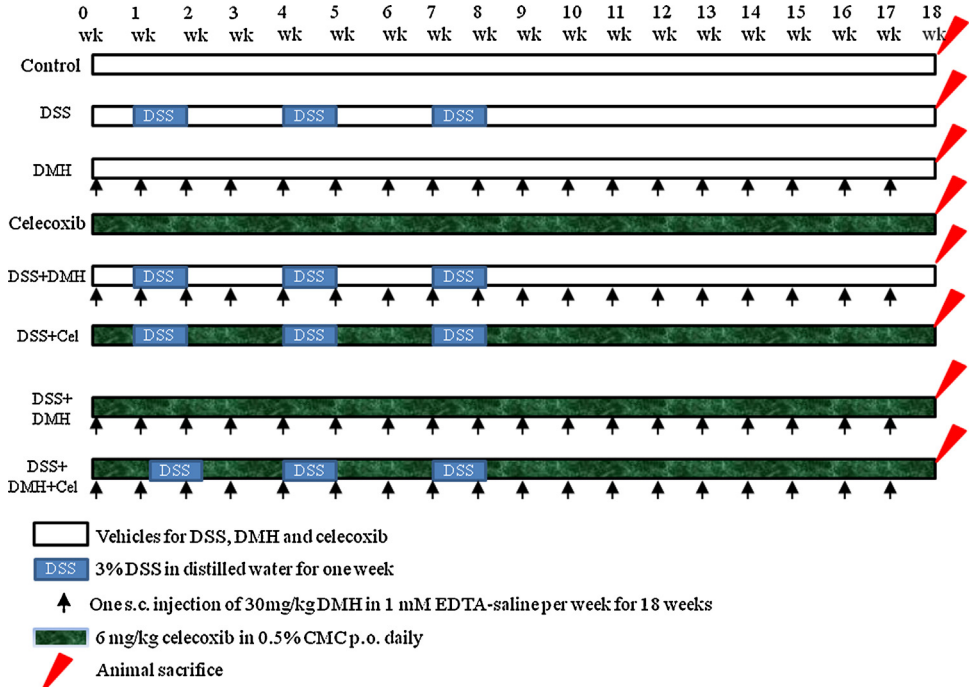

Fig. 1. Experimental design: control group is treated with the vehicle for DSS, DMH and DSS + DMH. The DSS group received three cycles of 3% DSS each for one week followed by two weeks of distilled water. The DMH group received one s.c. injection of 30 mg/kg DMH in 1 mM EDTA-saline per week for 18 weeks. The celecoxib group received 0.6 mg/kg celecoxib in 0.5% CMC p.o. daily. The other groups received different combinations of DSS, DMH and celecoxib.