Cancer Letters 328 (2013) 73–82

Contents lists available at SciVerse ScienceDirect

Cancer Letters

journal homepage: www.elsevier.com/locate/canlet

Celecoxib induces proliferation and Amphiregulin production in colon subepithelial myofibroblasts, activating erk1–2 signaling in synergy with EGFR

Roberto Benelli [a,*], Roberta Venè [b], Simona Minghelli [c], Sebastiano Carlone [b], Beatrice Gatteschi [d], Nicoletta Ferrari [b]

[a] Immunology Unit, IRCCS Azienda Ospedaliera Universitaria San Martino – Istituto Nazionale per la Ricerca sul Cancro (AOUSM-IST), Genoa, Italy
[b] Molecular Oncology & Angiogenesis Unit, AOUSM-IST, Genoa, Italy
[c] Clinical and Experimental Immunology Unit, Istituto Giannina Gaslini, Genoa, Italy
[d] Pathological Anatomy and Cytohistology Unit, AOUSM-IST, Genoa, Italy

ARTICLE INFO

Article history:
Received 20 July 2012
Received in revised form 12 September 2012
Accepted 12 September 2012

Keywords:
Myofibroblast
Colon
Celecoxib
COX-2
Amphiregulin
Erk1–2

ABSTRACT

The COX-2 inhibitor Celecoxib, tested in phase III trials for the prevention of sporadic colon adenomas, reduced the appearance of new adenomas, but was unable to affect the incidence of colon cancer. Moreover the 5 years follow-up showed that patients discontinuing Celecoxib treatment had an increased incidence of adenomas as compared to the placebo arm. In the APC(min/+) mouse model short term treatment with Celecoxib reduced gut adenomas, but a prolonged administration of the drug induced fibroblast activation and intestinal fibrosis with a final tumor burden. The way Celecoxib could directly activate human colon myofibroblasts (MF) has not yet been investigated.

We found that MF are activated by non toxic doses of Celecoxib. Celecoxib induces erk1–2 and Akt phosphorylation within 5′. This short term activation is apparently insufficient to cause phenotypic changes, but the contemporary triggering of EGFR causes an impressive synergic effect inducing MF proliferation and the neo-expression and release of Amphiregulin (AREG), a well known EGFR agonist involved in colon cancer progression. As a confirm to these observations, the erk inhibitor U0126 and the EGFR inhibitors Tyrphostin and Cetuximab were able to contrast AREG induction.

Our data provide evidence that Celecoxib directly activates MF empowering EGFR signaling. According to these results the association with EGFR (or erk1–2) inhibitors could abolish the off-target activity of Celecoxib, possibly extending the potential of this drug for colon cancer prevention.

1. Introduction

Colon cancer is characterized by a long life history, showing an increased risk of occurrence in over 50 people. Early lesions, like aberrant crypt foci and low grade adenomas, can remain quiescent for decades and only a low percentage of them will eventually evolve into cancer. This pattern of carcinogenesis makes this tumor particularly suitable for chemopreventive approaches able to postpone, or block cancer onset. COX-1 and 2 are key enzymes in inflammatory responses, controlling prostaglandins neo-synthesis. COX-2 has been linked to colon cancer progression as it results constitutively upregulated in this tumor. On these bases, several COX-1/2 inhibitors have been tested in chemopreventive settings both against familiar adenomatous polyposis (FAP) and sporadic colon cancer. FAP patients are particularly sensible to chemoprevention with aspirin and sulindac, showing the ability to strongly reduce adenoma occurrence [1]. Unfortunately, these non specific inhibitors of cyclooxygenases share the common side effect of causing ulcers. While COX-1 is stably expressed by target cells with physiologic roles, COX-2 is induced by inflammatory stimuli and is considered the true target for therapy [2]. For this reason a first generation of specific COX-2 inhibitors was developed. These drugs reduced the incidence of gastrointestinal hemorrhage, though an increased risk of stroke was found in patients treated for long times and at high dose [1]. Celecoxib finally gained a sufficient risk/benefit ratio, being approved for the chemoprevention of FAP [2]. Successive studies on sporadic colon cancer [3,4] indicated its possible extension to a larger cohort of patients, once overcome cardiac cytotoxicity.

Despite the efficacy in preventing the neo formation of adenomas, Celecoxib was unable to reduce the incidence of colon cancers in treated patients [5], moreover those patients discontinuing Celecoxib treatment showed an increased incidence of adenomas (1.66×) with a more aggressive phenotype, after 2 years [6]. As

* Corresponding author. Address: s.c. Immunologia, IRCCS Azienda Ospedaliera Universitaria San Martino – Istituto Nazionale per la Ricerca sul Cancro (AOUSM-IST), Largo Rosanna Benzi 10, 16132 Genoa, Italy. Tel.: +39 010 5737 234; fax: +39 010 5737 409.
E-mail address: roberto.benelli@istge.it (R. Benelli).

0304-3835/$ - see front matter © 2012 Elsevier Ireland Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.canlet.2012.09.008

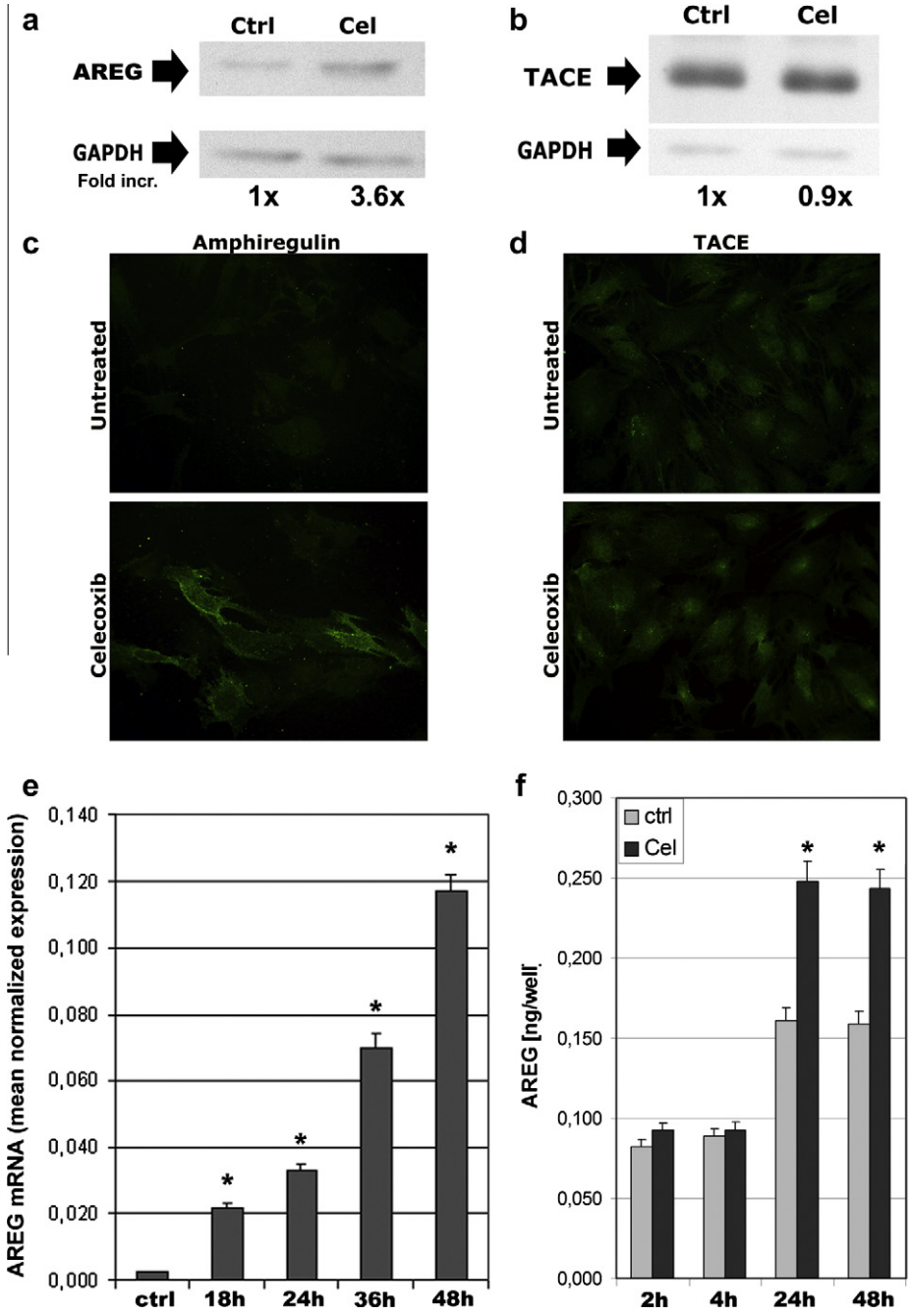

Fig. 1. Celecoxib induces AREG production in colon MF. MF in complete medium (DMEM 10% FCS) were treated with Celecoxib (50 μM) for 24 h (a–d) or as indicated (e and f). The western blot analysis (a) and immunofluorescence (c) of MF showed increased total and membrane-associated AREG. ADAM17/TACE was basally expressed by MF and not upregulated by Celecoxib as total protein (b), though it was accumulated in the cell membrane upon Celecoxib treatment (d). Relative intensity of WB lanes was obtained normalizing AREG and TACE signals over GAPDH controls, assuming untreated controls as baselines. The real time PCR analysis of AREG transcription showed a time dependent increase of its mRNA (e) (Statistical analysis. The *p* values ranged from 0.0117 for Cel vs. ctrl 18 h, to 0.0019 for Cel vs. ctrl 48 h.). This increase in mRNA was not paralleled by protein secretion (f), that reached a plateau starting from 24 h (Statistical analysis. Cel vs. ctrl 24 h $p = 0.021$; Cel vs. ctrl 48 h $p = 0.023$.).

COX-2 expression is increased through colon cancer progression [7–9] and Celecoxib is a specific COX-2 inhibitor, one should expect a significant therapeutic effect also against established cancer, without exacerbation upon drug discontinuation. These observations suggest that Celecoxib could exert an off-target activation of some cell populations of the gut, an hypothesis sustained by the observations of Bertagnolli *and coll.*, demonstrating the ability of chronic Celecoxib to activate gut myofibroblasts in the Min/(+) mice model [10–12].

To our knowledge a direct, stimulatory activity of Celecoxib on human colon myofibroblasts (MF) has never been described. We found that MF derived from both normal and tumoral mucosa can be activated by non toxic doses of Celecoxib. Sub-epithelial MFs are necessary for the physiology of colon crypts and their activation and proliferation accompanies and supports also colon carcinogenesis through the release of cytokines and proteases [13]. Celecoxib, when tested alone on quiescent MF, is able to induce erk1–2 and Akt phosphorylation within 3–5′. This short term activation ($\leqslant$30′) alone induces a weak but significant cell proliferation accompanied by the release of Amphiregulin (AREG). When MF are co-stimulated with EGF an impressive synergic effect on MF proliferation and AREG release is observed. AREG is an EGFR