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RESEARCH

# Pharmacogenomic study of EGF treatment in a rat DSS-induced colitis animal model

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**ABSTRACT**

Ulcerative colitis (UC) is a human chronic inflammatory disease of the colon and rectum, of partially characterized etiology. The Dextran Sulfate Sodium-induced colitis model in rats molecularly reproduces the human disease, aiding to evaluate therapeutic formulations aimed to repair the damage at the intestinal mucosa. Previous experiments have shown the effectiveness of rhEGF for healing colitis. However, the changes in the gene expression profile associated to that positive effect and its mechanism of action have been poorly studied. Therefore, this work was aimed to evaluate the colitis-related gene expression in the DSS model in rats, seven days after treatment with rhEGF formulated in pellet. Samples of distal colon tissue were taken from rats with DSS-induced colitis treated with rhEGF, followed by the extraction of total RNA. The gene expression of 46 genes was analyzed by quantitative PCR, those genes related to inflammation, oxidative stress, and cell differentiation and proliferation processes. The oral treatment with the rhEGF pellet formulation was shown effective, with more than 50 % of the analyzed genes related to inflammation and oxidative stress showing a statistically-significant decrease in their expression ($p = 0.05$). Moreover, a reverted expression profile was found for the genes involved in cell differentiation and proliferation processes in comparison with untreated animals. All this suggested the positive effect of the treatment with rhEGF formulated in pellet on the restoration of the intestinal epithelium, with the subsequent decrease in inflammation and oxidative stress.

*Keywords:* Ulcerative Colitis, rat model, sodium dodecyl sulphate, hrEGF, qPCR, oxidative stress, inflammation, cell differentiation and proliferation

**RESUMEN**

**Estudio farmacogenómico del tratamiento con FCE en un modelo animal de colitis ulcerativa inducida por DSS en ratas.** La colitis ulcerativa (CU) es una enfermedad inflamatoria crónica humana del colon y el recto, que se reproduce molecularmente en el modelo de rata mediante su inducción con dextrano sulfato de sodio (DSS). El ensayo de formulaciones terapéuticas orientadas a reparar el daño en la mucosa intestinal ha demostrado la eficacia del factor de crecimiento epidémico humano recombinante (FCEhr) para curar la enfermedad. Sin embargo, los cambios en el perfil de expresión de los genes asociados a dicho efecto positivo y su mecanismo de acción no han sido caracterizados. En este trabajo se evaluó la expresión génica vinculada a la patogénesis de la CU en el modelo de DSS en ratas, siete días después de administrar el FCEhr por vía oral formulado en pellet. Se tomó muestras del colon distal de los animales con CU tratados y sin tratar con FCEhr, y se les extrajo el ARN total. Se analizó la expresión de 46 genes relacionados con la inflamación, el estrés oxidativo y los procesos de diferenciación y proliferación celulares, mediante PCR cuantitativo. El tratamiento fue efectivo y redujo significativamente ($p < 0.05$) la expresión en más del 50 % de los genes vinculados a la inflamación y el estrés oxidativo en los animales tratados con respecto a los no tratados. También se revirtió el patrón de expresión patológica de los genes asociados a la diferenciación y la proliferación celulares. Esto indicó el efecto positivo del tratamiento en la restitución del epitelio intestinal, al disminuir la inflamación y el estrés oxidativo de la CU.

*Palabras clave:* Colitis ulcerativa, modelo en ratas, dextrana sulfato de sodio, FCEhr, PCR cuantitativo, estrés oxidativo, inflamación, diferenciación y proliferación celular

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## Introduction

The inflammatory bowel disease (IBD) includes Crohn's disease and ulcerative colitis (UC), two complex diseases of increasing incidence and prevalence worldwide among the human population [1, 2]. IBD are regarded as triggered by multiple factors and of incompletely characterized etiology. There has been hypothesized that IBD result from a deregulation of the intestinal barrier, followed by a pathological immune activation against viral or bacterial antigens that, thereby, leads to inflammation and oxidative stress mediated damage of colon mucosa [3-5]..

The intestinal epithelium acts as a physical barrier which blocks gut microbial flora from penetrating into the intestinal submucosa [6]. The intestinal mucosa is physiologically subjected to a mechanically and microbiologically hostile environment, with a cell turnover every 5-7 days from intestinal stem cells (ISC) located at the bottom of intestinal crypts [7].

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These generate the so-called transit amplifying (TA) cells which after few other cell divisions differentiate in adult epithelial cells. The Wnt/β-Catenin pathway is among the principal differentiation routes of mucosal intestinal cells [8]. It is known that the active proliferation process of TA cells is specifically stimulated by the epithelial growth factor (EGF) [9].

The EGF has been used as enemas successfully to treat distant UC [10], and to heal gastrointestinal lesions when orally administered [11, 12]. However, none of those studies further elucidated the molecular events mediating the EGF positive effects on the gastro-duodenal mucosa or the mechanisms underlying it in UC. Previously, our group addressed the molecular characterization of a model of human colitis in rats treated with dextran sodium sulfate (DSS) [13]. Therefore, this work was aimed to evaluate the expression of 46 genes related to inflammation, oxidative stress, epithelium integrity, and cell differentiation and proliferation processes in rats with DSS-induced experimental colitis, in response to treatment with the recombinant human EGF (rhEGF) formulated in pellets (Patent number: EP2533758B1) [14].

## Materials and methods

### Experimental design

The UC model in rats was established as previously reported, by administering 8 % DSS for six days [15]. The animals were randomly distributed among experimental groups, in compliance with standard guidelines for care and handling of laboratory animals [16]. Two groups of five animals each were used as healthy control (Group I), and untreated DSS-induced colitis biomodel control (Group II). The other four animal groups, eight animals each with established DSS-induced colitis were subjected to experimental treatments, starting on day 6 and for seven days. Subsequently on day 14, animals were sacrificed and biopsies taken as described. Treatments were: Group III, rhEGF-vehicle; Group IV: rhEGF 125 µg/pellet/day; Group V: rhEGF 250 µg/pellet/day; Group IV: rhEGF 500 µg/pellet/day.

### Histology sample evaluation

Samples taken were characterized by histology through a semiquantitative scale structured from the scale described by FitzGerald *et al*. [17]. A set of essential histology variables was evaluated, comprising: severity of inflammation (SI), extension of inflammation (EI), involved percentage (IP), crypt damage (CD), and a total histology score (THS) was calculated. The mean histology damage mean values and their standard deviation were determined for each group. Numerical results were statistically compared and graphically represented with the aid of the statistical GraphPad Prism program (www.graphpad.com; version 5.03).

The anatomopathological results of groups I and II were used to corroborate the establishment of colitis in rats as previously reported [17], and biopsies samples from groups III-VI were further analyzed for differential gene expression to study the effect of rhEGF pellets on DSS-induced colitis.

### RNA extraction

Distant colonic samples of approximately 30 mg each from five animals of experimental groups III-VI were processed using the Tissue Lyser (Qiagen, Hilden, Germany) equipment. Total RNA was purified with the RNeasy® Plus (QIAGEN GmbH, Germany) reagents kit by the Quiacube platform. RNA concentration and integrity were determined with NanoDrop® (NanoDrop Technologies, USA) and Bioanalyzer (Agilent, 2100, USA) equipment.

### Complementary DNA strand synthesis

Complementary DNA (cDNA) was synthesized starting from 1 µg of total RNA by using the kit Superscript III First-Strand Synthesis Supermix for RT-PCR (Invitrogen Technologies, Carlsbad, California, USA), following the manufacturer's instructions.

### Quantitative PCR and genomic tools

Primers for the quantitative PCR assay were designed using the Primer3 software (http://www-genome.wi.mit.edu/genome_software/other/primer3.html), for 22-mer primers with a 50 % average G+C content (Table 1) [18]. Due to their stable transcriptional levels in the selected samples, *GAPDH*, *MAP2K5*, *MAPK6*, *RPL13A* and *YWHAZ* genes were selected as reference genes, with the aid of the GeNorm software [19]. Similarly, rPCR efficiency was estimated with the aid of the LingReg program (version 11.3, 2009; Amsterdam, Netherlands) [19]. qPCR reactions were prepared on 20-µL total reaction volume containing 10 µL of ABsolute qPCR SYBR® Green Mix (Thermo Scientific, USA), 6 µL of primers (70 nM) and 4 µL of cDNA diluted 25 times.

Reactions were run in a CapitalBio RT-Cycler001 (CapitalBio Co., Ltd., Beijing, China) equipment, by incubation at 95 °C for 15 min, followed by 40 cycles at 95 °C for 15 s, 60°C for 30 s and 72 °C for 30 s. qPCR results were analyzed using the CapitalBio RT-CyclerVersion 2.001 (CapitalBio Co., Ltd., Beijing, China). The relative quantification of the gene expression was performed with the REST 2009 v2.0.13 software (Qiagen GmbH, Germany).

### qPCR of EGF pathway genes

Gene expression of 46 genes related to inflammation, oxidative stress and cell differentiation and proliferation processes were studied by qPCR (Table). The qPCR reactions from samples of groups III, V and VI were analyzed, with three replicates per biological sample. For this, a commercially available 96-well plate containing all the primers for the amplification of the EGF/PDGF signaling pathway (cat: PARN040Z, Qiagen, Germany) was processed with the Real time PCR protocol for RT2 profiler PCR array format C, in a Light Cycler 480 (Roche, Germany) equipment.

### Statistic analysis

Total histology damage was compared among groups by a two-tailed Student's t test for non-paired samples. Standard deviations of the mean were calculated for histology damage (n = 5). Differential expression analysis was carried out with the aid of the statistical analysis provided by the REST software (version 2.0.13, 2009; Qiagen GmbH, Germany). Differences were regarded as statistically significant ($p < 0.05$).

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Table 1. qPCR primers for the analysis of inflammation, oxidative stress and cell differentiation of intestinal epitelial cells, and reference genes in DSS-induced ulcerative colitis in rats treated with recombinant human epidermal growth factor in pellets*

| Biological function | Gene name | Accession number | qPCR primers Forward (5´-3´) | Reverse (5´-3´) |
|---|---|---|---|---|
| Inflammation | *TNFα* | NM_012675.3 | TACACTGGCCCGAGGCAACACA | AGACACCGCCTGGAGTTCTGGA |
| | *IL-6* | NM_012589.2 | GATACCACCCACAACAGACCAG | CAGTGCATCATCGCTGTTCATA |
| | *IL-1b* | NM_031512.2 | ACGTGTGTTTCCCTCCCTGCCT | TCGACAATGCTGCCTCGTGACC |
| | *IL-17* | NM_001106897.1 | GTTCCACTTCACCCTGGACTCT | GAATTCATGTGGTGGTCCAACT |
| | *IL-13* | NM_002188.2 | AGCTTATCGAGGAGCTGAGCAA | CTGGAGATGTTGGTCAGGGATT |
| | *IFNγ* | NM_138880.2 | TGCTACACGCCGCGTCTTGGTT | TGAGTGTGCCTTGGCAGTAACAGCC |
| | *PTGS2* | NM_000963.3 | ACAGATTGCTGGCCGGGTTGCT | TGGAGGCCTTTGCCACTGCTTGT |
| | *NF-κB1* | NM_001165412.1 | GATGGGACGACACCTCTACACA | CCAAGAGTCGTCCAGGTCATAG |
| Oxidative stress | *CCL12* | NM_001105822.1 | AATCACAAGCAGCCAGTGTCCCCG | ACCCACTTCTCCTTGGGGTCAGCA |
| | *CXCL2* | NM_053647.1 | GCAAGCTCCCTCCTGTGCTCAA | CACTGGCCACAACAACCCCTGT |
| | *CAT* | NM_001752.3 | AAGCGCTTCAACAGTGCTAATG | AGCTGAGCATCTTTCAGGTGGT |
| | *GPX1* | NM_030826.3 | GGGACTACACCGAAATGAATGA | GGTCGGACATACTTGAGGGAAT |
| | *INOS* | NM_000625.4 | GGAAGAAATGCAGGAGATGGTC | TTTCTGCAGGATGTCTTGAACG |
| | *SOD2* | NM_002083.3 | GGAACAACTACCCGGGACTACA | GGCTGTTCAGGATCTCCTCATT |
| | *NFE2L2* | NM_031789.2 | TACAGCAGGACATGGAGCAAGT | ATCTCTGTCAGTGTGGCCTCTG |
| Transcription factor | *GFI1* | NM_001127215.1 | GTGAGCCTGGAGCAACACAAG | CGGGTATCCGAGTGAATGAGTA |
| | *KLF4* | NM_001314052.1 | AAAAGAACAGCCACCCACACTT | TCCCAGTCACAGTGGTAAGGTT |
| | *ELF3* | NM_001114309.1 | CCCGAAAGCTGAGCAAAGAATA | ATTTCATGAGGCCTTCGTTGAG |
| Reference genes | *GAPDH* | NM_001256799.2 | CAAGTTCAACGGCACAGTCAAGG | ACCAGCATCATCACCCCATTTGATCTTG |
| | *MAP2K5* | NM_001206804.1 | TTGTAAACACAAGCGGACAGGT | CTTTCGGGTGCCATATAAGCATGATCTTG |
| | *MAPK6* | NM_002748.3 | TTAGTCGGGAAGCACTGGATTT | CCGTTGGGAAAGAGTAGATGCT |
| | *RPL13A* | NM_001270491.1 | TCCGAGCCCCAAGCCGCATTTT | AGCAGGGACCACCATCCGCTTT |
| | *YWHAZ* | NM_001135699.1 | TTGGTGTGTGCTGGCGGGGAAT | TGTGCACGCAGACACACGGTCT |

* The commercial plate catalogue number PARN040Z (Qiagen, Germany) was used for analyzing the expression of genes of the Jak/Stat signaling pathway (*JAK1*, Accession number NM_053466; *STAT3*, NM_012747; *STAT5a*, NM_017064), Catenin β pathway (*CSNK2A1*, NM_053824; *CSNK2B*, NM_031021) and cell proliferation genes (*BCAR1*, NM_012931; *HRAS*, NM_001098241; *RAF1*, NM_012639; *MAPK1*, NM_053842; *MAPK3*, NM_017347; *EGFR*, NM_031507; *GRB2*, NM_030846; *GAB1*, NM_001108444; *EPS8*, XM_232499; *NCK2*, NM_001108216; *NRAS*, NM_080766; *KRAS*, NM_031515; *RHOA*, NM_057132; *RASA1*, NM_013135; *RAP1A*, NM_001005765; *MAPK8*, NM_053829; *MAPK9*, NM_017322; *MAPK10*, NM_012806; *MKNK1*, NM_001044267; *MAP2K1*, NM_031643; *MAP2K4*, NM_001030023; *MAP2K7*, NM_001025425; *MAP3K2*, NM_138503).

## Results and discussion

### Histology score

After sacrificing the animals, their distal colon were histopathologically diagnosed to confirm that the colitis model was established and, secondly, the effect of the pellet-rhEGF on the colonic intestinal epithelium. The THS calculated from SI, EI, IP and CD variables evidenced a colonic mucosa histological deterioration in response to DSS, confirming the reproduction of the UC model previously reported (Figure 1) [17].

Subsequently, the therapeutic effect of the rhEGF pellet on the disease at colon mucosa was assessed. For this, histological samples from groups IV-VI corresponding to treatment with rhEGF pellets for seven days at dosages of 125, 250 or 500 μg/pellet/day, respectively, were compared to those in animals of Group III receiving the pellet formulation vehicle. As shown, a recovery of damaged mucosa was found in animals from groups V and VI, corresponding to the 250 or 500 μg/pellet/day dosages, with statistically significant reduction in the THS index, indicating a tissue improvement at the distal colon. There were no statistically significant results in animals from group IV (125 μg/pellet/day). Moreover, the 250 or 500 μg/pellet/day dosages performed similar, with no statistically significant difference between them (Figure 1). Hence, the samples from both groups were jointly analyzed (n = 10) against samples from animals in group III (rhEGF pellet vehicle) as control group, for the differential expression of genes involved in inflammation, oxidative stress and cell differentiation and proliferation processes.

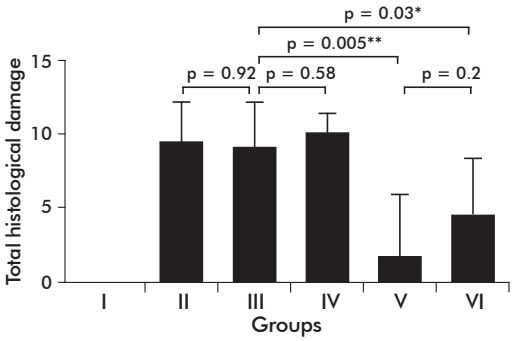


Figure 1. Histological damage in a DSS-induced ulcerative colitis model in rats and the effect of treatment with three dosages of recombinant human epidermal growth factor (rhEGF) in pellets. The histological damage results were compared by a two-tailed Student t test for non-paired samples. Groups: I, healthy rats; II, 8 % DSS-induced ulcerative colitis rats; III, ulcerative colitis rats treated with pellet formulation without recombinant human epidermal growth factor (rhEGF); IV-VI, rats treated with rhEGF pellet formulations at dosages 125, 250 or 500 μg/pellet/day, respectively. Error bars stand for half the standard deviation of the mean (n = 5). Statistically and very significant differences are indicated (* $p < 0.05$ and ** $p < 0.01$, respectively).

### Differential gene expression study

Compared to rats with DSS-induced UC and treated with rhEGF pellet vehicle, the animals treated with 250 or 500 µg/pellet/day showed decreased expression of genes coding for pro-inflammatory cytokines and oxidative stress. Such a response was statistically significant for genes *TNFα*, *IL-1b*, *IL-6*, *CCL2*, *CXCL2*, *PTGS2*, *GPX1*, *INOS* and *SOD2* (Table 2, Figure 2). Consistently, genes related to the Jack-Stat pathway and the transcriptional factor NF-κβ1, involved in cytokine signal transmission and in the upregulation of proinflammatory cytokines, respectively, also showed lower expression [20, 21], statistically significant for the Jak1 receptor and NF-κβ1 (Table 2, Figure 2). These results provide molecular evidences on a downregulation of both, the inflammation and the oxidative stress processes, by the treatment with the rhEGF pellet.

The intestinal tissue physiology is characterized by the constant turnover of mucosal cells from intestinal stem cells (ISCs). The Wnt/β-Catenin is a key pathway for the stimulation of intestinal cells differentiation, and the Casein kinase (CNK) enzyme that upregulates this pathway [8, 22]. In this sense, the expression levels of the *CNK2a1* and *CNK2b* genes were found raised, the second with statistically significant differences when compared to levels found in animals treated with the pellet formulation vehicle. This could be indicative of the activation of the Wnt/β-Catenin pathway. Furthermore, the expression of two other β-Catenin-activated transcriptional factors' genes was found potentiated, statistically significant for KLF4 (Table 2, Figure 3A). KLF4 is involved in the formation of caliciform cells, responsible for the production of Mucin2 and Trefoild Factor, which are structural components of the protective barrier of the intestinal epithelium [8, 23]. Noteworthy, these genes involved in cell proliferation in animals treated with rhEGF pellet at 250 and 500 µg/pellet/day dosages showed an opposite expression performance to previous results reported by Roca *et al*. [13] in animals treated with DSS. Nevertheless, it is advisable to corroborate the activation of the Wnt/β-Catenin pathway, by inmunohistochemical assay in order to corroborated the nuclear localization of β-Catenin.

rhEGF maintains an active proliferation of intestinal epithelial cells (IECs) [9]. More than half of the EGF pathway genes showed over 50 % increased expression, five of them significantly: *BCAR1*, *HRAS*, *RAFA1*, *MAPK1* and *MAPK3*. Contradictorily, the expression of the EGF receptor (EGFR) gene was reduced, maybe as target of the NF-κB transcriptional factor. Moreover, such a reduction could be relative due to the experimental setting, since comparison was established between animals treated with rhEGF, with lower inflammation and *NF-κB* expression, and animals treated with placebo.

Besides, more than 70 % of the 23 genes tested relating to the EGF pathway showed an regulation indicating its activation, an effect opposite to that previously reported by Roca *et al*. in this DSS-induced UC animal model [13] (Table 2, Figure 3B).

Noteworthy, UC is a cancer-related condition, since the incidence of UC-associated cancer (UCC) increases notably with UC progression [24]. Historically, growth factors have been regarded as cancer-related, together with clinical evidences on EGFR over-expression in most epithelial cell tumors as a poorer prognostic marker [25]. This has led to concerns on EGF use as promoting UC-associated cancer, further limiting UC therapeutic approaches using it [26]. However, the main mechanism linking inflammation to pro-neoplasic genetic alterations is oxidative stress. It is triggered and maintained mainly by cells of the innate immune system, like macrophages and granulocytes. Those cells release several reactive oxygen and nitrogen species physiologically involved in the eradication of mucosal pathogens, but pathological leading to intestinal damage, DNA mutation and damaging cellular proteins and lipids [24]. Consequently, it has been demonstrated that in direct or indirect inhibition of oxidative stress processes

Table 2. Differential gene expression levels in colonic epithelium samples of rats with DSS-induced ulcerative colitis treated with either 250 or 500 µg/pellet/day of recombinant human epidermal growth factor (rhEGF) in pellets, compared to untreated animals

| Inflammation and OS | | | Cell differentiation | | | Cell proliferation | | |
|---|---|---|---|---|---|---|---|---|
| Gene | Change level | p value | Gene | Change level | p value | Gene | Change level | p value |
| *TNFα* | –4.10 | 0.000** | *KLF4* | 1.944 | 0.003** | *EGFR* | –1.24 | 0.11 |
| *IL-1b* | –3.50 | 0.002** | *CSNK2B* | 1.260 | 0.00 ** | *GRB2* | –1.11 | 0.31 |
| *IL-6* | –5.20 | 0.014* | *GFI1* | 1.664 | 0.231 | *GAB1* | –1.05 | 0.81 |
| *CCL12* | –2.56 | 0.002** | *ELF3* | 1.067 | 0.817 | *EPS8* | 1.36 | 0.21 |
| *CXCL2* | –5.88 | 0.009** | *CSNK2A1* | 1.090 | 0.400 | *NCK2* | 1.17 | 0.72 |
| *PTGS2* | –4.10 | 0.000** | | | | *NRAS* | –1.02 | 0.69 |
| *GPX1* | –1.53 | 0.008** | | | | *KRAS* | 1.07 | 0.33 |
| *INOS* | –6.25 | 0.007** | | | | *RHOA* | 1.01 | 0.68 |
| *SOD2* | –2.22 | 0.016* | | | | *RASA1* | 1.08 | 0.46 |
| *JAK1* | –1.29 | 0.00 ** | | | | *RAP1A* | –1.05 | 0.76 |
| *IL-17* | –2.00 | 0.172 | Cell proliferation | | | *MAPK8* | 1.05 | 0.59 |
| *IL-13* | 1.47 | 0.305 | Gene | Change level | p value | *MAPK9* | 1.14 | 0.18 |
| *IFNγ* | –1.69 | 0.165 | | | | *MAPK10* | –1.11 | 0.97 |
| *CAT* | –1.21 | 0.277 | *BCAR1* | 1.53 | 0.04* | *MKNK1* | –1.17 | 0.09 |
| *NFE212* | –1.14 | 0.455 | *HRAS* | 1.53 | 0.00** | *MAP2K1* | –1.03 | 0.90 |
| *NF-κB1* | –1.43 | 0.007** | *RAF1* | 1.11 | 0.03* | *MAP2K4* | 1.08 | 0.73 |
| *STAT3* | –1.22 | 0.110 | *MAPK1* | 1.17 | 0.03* | *MAP2K7* | –1.10 | 0.55 |
| *STAT5a* | –1.29 | 0.210 | *MAPK3* | 1.54 | 0.00** | *MAP3K2* | 1.01 | 0.72 |

*, ** Significant and highly significant statistical differences (* $p < 0.05$ and ** $p < 0.01$, respectively). Values stand of the expression change levels of each gene in rhEGF-Pellet-treated animals with 250 or 500 µg/pellet/day compared to placebo-treated rats.

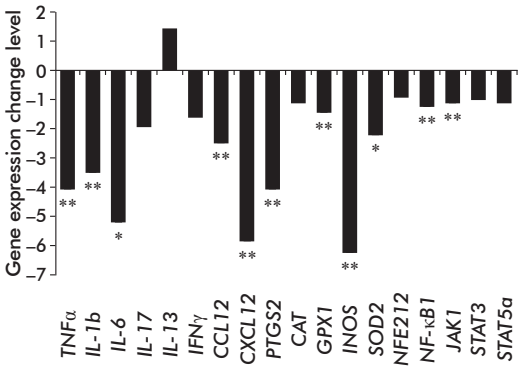


Figure 2. Changes of expression levels for inflammation and oxidative stress genes detected by qPCR in colonic biopsies in rats with DSS-induced ulcerative colitis, treated with pellets of rhEGF. Values stand of the expression change levels of each gene in rhEGF pellet-treated animals with 250 or 500 µg/pellet/day compared to placebo-treated rats. Statistically significant differences are indicated (* $p < 0.05$, ** $p < 0.01$).

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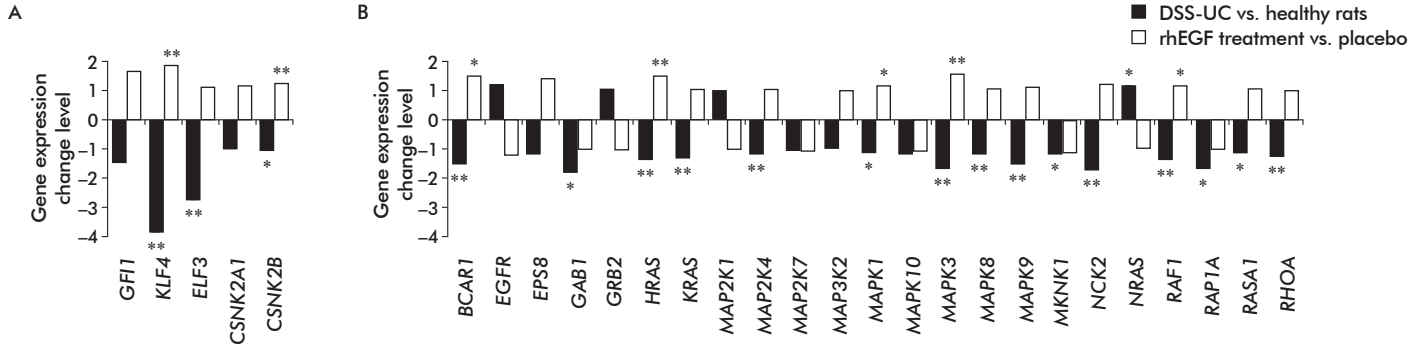


Figure 3. Changes of expression levels for proliferation and differentiation of intestinal cells genes detected by qPCR in colonic biopsies of rats with DSS-induced ulcerative colitis, treated with pellets of recombinant human epidermal growth factor (rhEGF). A) Wnt/β-Catenin pathway genes. B) Epidermal growth factor pathway genes. Values stand of the expression change levels of each gene for rhEGF-Pellet-treated animals with 250 or 500 μg/pellet/day compared to placebo-treated rats (white bars) or DSS-UC vs. healthy rats (black bars; data taken from Roca *et al*. Bionatura. 2018; 3(2):577-81). Statistically significant differences are indicated (* $p < 0.05$, ** $p < 0.01$).

interrupts DNA damage and decreases the incidence of intestinal tumors [27]. Similarly, there was reported that a decreased activation of NF-κB significantly protects against tumor development by persistent inflammation [28], in experimental models of UCC, as well as IL-6, IL-1b, TNFα, and the STAT3 transcription factor [27].

In this work, the previously mentioned genes significantly decreased its expression upon treatment with rhEGF, possibly suggesting an lowered risk for UCC, in agreement with previous animal studies indicating that EGF-pathway activation could lead to a long term reduction of UCC [29, 30].

Hence, the therapeutic rhEGF pharmaceutical formulation, equivalent to that used in our work in the DSS-induced UC model, should have to be carefully evaluated for biosafety prior to human application. Overall, this is the first Omics study shedding light into the mechanism of action of rhEGF in UC. Our results indicate that the rhEGF formulation used induces proliferative and differentiation processes in colonic epithelial cells (Figure 4), which could favor the restoration of the colonic epithelial barrier and homeostasis.

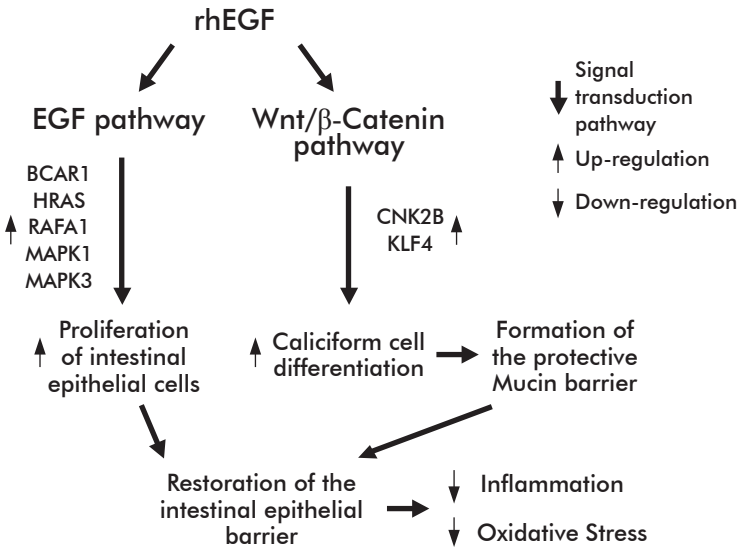


Figure 4. Putative mechanism of action for the treatment of ulcerative colitis with recombinan human epidermal growth factor (rhEGF) in pellet formulations. Experimental results supporting the proposed model were obtained in the DSS-induced ulcerative colitis model in rats.

## Conclusions

The treatment of rats suffering from DSS-induced UC with rhEGF-pellet formulations led to a change in the expression profile of genes associated to IECs proliferation and differentiation. This suggests that a positive effect on the restoration of intestinal epithelium, with subsequent decrease in inflammation and OS. These results support the future evaluation of rhEGF pellet formulations for treating UC on its initial phases, limiting its progression and chronification.

## Conflicts of interest statement

The authors declare that there are no conflicts of interest.

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