

TGF- β receptor I inhibitor may restrict the induction of EMT in inflamed intestinal epithelial cells

Mahsa Ghorbaninejad¹, Meghdad Abdollahpour-Alitappeh², Shabnam Shahrokh³, Sara Fayazzadeh⁴, Hamid Asadzadeh-Aghdaei¹, Anna Meyfour^{1*}

¹Basic and Molecular Epidemiology of Gastrointestinal Disorders Research Center, Research Institute for Gastroenterology and Liver Diseases, Shahid Beheshti University of Medical Sciences, Tehran, Iran

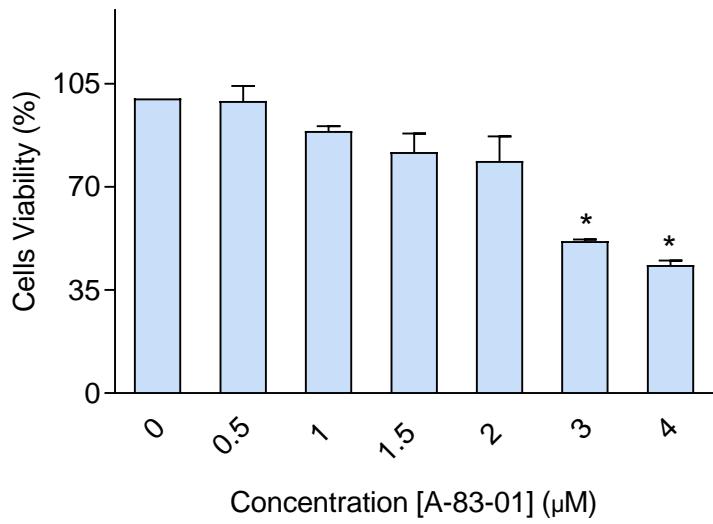
²Cellular and Molecular Biology Research Center, Larestan University of Medical Sciences, Larestan, Iran

³Gastroenterology and Liver Diseases Research Center, Research Institute for Gastroenterology and Liver Diseases, Shahid Beheshti University of Medical Sciences, Tehran, Iran

⁴Bioinformatics and Computational Omics Lab (BioCOOL), Department of Biophysics, Faculty of Biological Sciences, Tarbiat Modares University, Tehran, Iran

Corresponding author:

Anna Meyfour, Ph.D., Research Institute for Gastroenterology and Liver Diseases, Shahid Beheshti University of Medical Sciences, Arabi Ave., Daneshjoo Blvd., Velenjak, Tehran, Iran. Postal Code: 1985717413, Tel: +98 21 22432521. Email: a.meyfour@sbmu.ac.ir



Supplementary Fig1. The cells were treated with different concentrations of TGF- β (0, 0.5, 1, 1.5, 2, 3 and 4 μ M, respectively) for 24 hours, and the cell viability was determined by MTS assay. Data are expressed as the mean \pm SEM ($n=3$) and significant differences are highlighted: * $p<0.05$.

Supplementary Table 1. List of primers used for quantitative real time (qRT)-PCR

Gene name	Primer sequence 5' → 3'	Product size
GAPDH	F: CTCATTCCTGGTATGACAACGA R: CTTCCTCTTGCTCTTGCT	121
OCCLUDIN	F: CCACGCCGGTCTGAAGTGG R: TCACAGGACTCGCCGCCAGT	199
CLAUDIN7	F: AGCTGCAAAATGTACGACTCG R: GGAGACCACCATTAGGGCTC	75
ZO-1	F: CGGT CCTCTGAGCCTGTAAG R: GGATCTACATGCGACGACAA	371
VIMENTIN	F: AAACTTAGGGCGCTCTTGT R: TGAGGGCTCCTAGCGGTTA	163
CDH1	F: CGAGAGCTACACGTTACCGG R: GGGTGTGAGGGAAAAATAGG	119
CDH2	F: TCAGGC GTCTGTAGAGGTT R: ATGCACATCCTCGATAAGACTG	94
SNAI1	F: CCAGAGTTTACCTTCCAGCA R: GATGAGCATTGGCAGCGA	102
SNAI2	F: AACTACAGCGAACTGGACAC R: GGATCTCTGGTTGTGGTATGAC	91
CTNNB1	F: CATCTACACAGTTGATGCTGCT R: GCAGTTTGTCAGTT CAGGGA	151
ACTA2	F: AAAAGACAGCTACGTGGGTGA R: GCCATGTTCTATCGGGTACTTC	76
TGF-β	F: CCCAGCATCTGCAAAGCTC R: GTCAATGTACAGCTGCCGCA	101
SMAD4	F: CTCATGTGATCTATGCCGTC R: AGGTGATACAACCTCGTTCTAGT	146
GAPDH	F: GACTTCAACAGCAACTCCCAC R: TCCACCACCTGTTGCTGTA	125

Supplementary Table 2. Detail information about the Antibody used for western blot.

Antibody	Cat no.	Company	Dilution
Vimentin	ab71144	Abcam	1:2500
E-Cadherin	ab231303	Abcam	1:1000
β -Catenin	SC7963	Santa cruz	1:1000
α -SMA	MA5-15871	Invitrogen	1:1000
p-SMAD2	E-AB-21040	Elabscience	1:1000
p-SMAD3	E-AB-21040	Elabscience	1:1000
p-AKT	40S85	Cell signaling	1:1000
RPS6	MAB54361	R&D	1:500
β -Actin	60008-1-Ig	Proteintech	1:20000

Supplementary Table 3. Demographic characteristics of patients included in the study.

CD: Crohn's disease, UC: Ulcerative colitis, BMI: Body mass index

Variable	Patients		Healthy Controls
	UC	CD	
Number of patients	20	16	24
Age (year)	38.50±14.58	41.75±12.91	39.08±14.20
Gender			
- Male	68%	75%	50%
- Female	32%	25%	50%
BMI (kg/m^2)	27.49±5.27	26.12±5.92	25.94±4.53
Family history	0	0	0
History of surgery	0	0	0
Smoking status			
- Smoker	3 (20%)	7 (53.84%)	2 (10%)
- Non-smoker	12 (80%)	6 (46.16%)	18 (90%)
Disease duration (month)	58±32.01	59±31.34	0