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LETTER TO THE EDITOR

# Transforming Growth Factor Beta 2 Inhibits Growth and Proliferation Potential of Smad4 and p53 Mutated Human Colon Adenocarcinoma Cells

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To the Editor

Colon cancer is the most frequently diagnosed cancer and the second leading contributors of cancer related deaths in the United States [1]. The American cancer society estimated that colon cancer causes about 49,190 deaths in 2016 [2]. Although current therapeutics have proved to be clinically favourable for patients who were diagnosed at early stages, development of multi-drug resistance and various side effects remains the potential obstacle for advanced stages of colon cancer [3]. Recent data suggests that the 5 year survival rate for advanced stage colon cancer is about 5% with increased mortality rate of more than 663,000 deaths annually worldwide [2, 3]. Molecular evidence confirmed that transforming growth factor beta (TGF-β) plays a crucial role in the metastatic progression of colon cancer and multi-drug resistance [4].

TGF-β regulates the expression of a plethora of genes involved in fundamental cellular processes such as cell proliferation, differentiation, apoptosis, and immune response. Currently three isoforms are identified in mammals including TGF-β1, TGF-β2 and TGF-β3 [5]. TGF-β exerts its effects by trans-signaling through ubiquitously expressed TGF-β receptors (TβRI and TβRII) at the cell surface. Following interaction with TGF-β, TGF-β receptors initiates Smad2/3/4 complex formation which subsequently translocates into the nucleus to regulate the expression of TGF-β responsive genes [6, 7]. TGF-β exhibits a dual role in colon cancer, initially it acts as a tumor suppressor, and however at the advanced stage it promotes cell proliferation, migration and metastasis [8]. The strongest evidences have suggested that alteration of TGF-β signaling pathway may contribute to the colon cancer progression [8, 9]. In addition, evidence have suggested that loss of Smad4 and p53 functions are important for the loss of TGF-β mediated tumor suppressor role in colon cancer [9, 10]. Furthermore, TGF-β has been shown to activate Smad independent extracellular signal–regulated kinases (ERK) pathway which are important for its pro-oncogenic activities [10]. Collectively, pharmacological inhibition of TGF-β mediated signaling cascade has emerged as one of the potential target for advanced colon cancer patients.

Previous studies have shown that the expression of TGF-β1 is upregulated in the early stages of colon cancer and inhibited proliferation and induced apoptosis [11]. However, in late stages of colon cancer, TGF-β1 is often overexpressed and found that the increased expression of TGF-β1 correlated with invasion and metastasis [12, 13]. Moreover, in advanced stages of colon cancer an increased expression of TGF-β2 was observed which results in worsening of the disease [14]. In addition, one recent study has found that TGF-β1 and TGF-β2 increases the invasive and migration potential of colon cancer cells [15]. Taken together, TGF-β1 and TGF-β2 plays a crucial role in colon cancer invasion and metastasis. However, the effect of TGF-β2 on the proliferation of Smad4 and p53 mutated colon adenocarcinoma cells still remains elusive. We analyzed the effect of TGF-β2 on the proliferation potential of Smad4 and p53 mutated human colon adenocarcinoma cell line HT-29.

In this study, we used HT-29 human colon adenocarcinoma cells which express mutated form of Smad4 and p53 proteins, were treated with 5 ng/ml of TGF-β2 and cellular viability and proliferation potential were assessed

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by trypan blue exclusion assay and 3-(4,5-dimethylthiazol-2-yl)-2,5- diphenyltetrrazolium bromide (MTT) assay as described previously [16]. After, detection of the effect of TGF-β2 on the cell viability and proliferation potential of HT-29 cells, lactate dehydrogenase (LDH) cell cytotoxicity assay was performed. On the trypan blue exclusion assay, we observed that TGF-β2 significantly inhibited the cell viability of HT-29 cells in a time-dependent manner. In addition, 57 to 65% reduction in cell viability was obtained at 96 and 120 h of TGF-β2 treatment (Fig. 1a). The anti-proliferative effect of TGF-β2 on HT-29 cells was detected by MTT assay and found that 58 to 61% reduction in cell proliferation of HT-29 cells was observed at 96 and 120 h of TGF-β2 treatment (Fig. 1b).

In addition, we did not observe any significant difference on the level of LDH release in the medium of TGF-β2 treated HT-29 cells when compared with untreated cells. This results confirmed that TGF-β2 did not showed cytotoxic effects to the HT-29 cells (Fig. 1c). We finally repeated MTT assay to further assess the time period at which TGF-β2 treatment exhibits maximal inhibitory effect on the cell proliferation of HT-29 cells. Interestingly, 73 to 98% reduction in the cell proliferation was observed upon TGF-β2 treatment for 20 and 30 days (Fig. 1d). In the present study, we report that TGF-β2 inhibited cell viability and proliferation potential of Smad4 and p53 mutated human colon adenocarcinoma cell line HT-29. In summary, our data indicates that continuous TGF-β2 treatment displays a tumor suppressor effect against Smad4 and p53 mutated colon adenocarcinoma. However, additional experimental studies at the molecular level are needed to further verify these observations. Present study may provide a strong foundation for further studies on the role of TGF-β2 in colon adenocarcinoma.

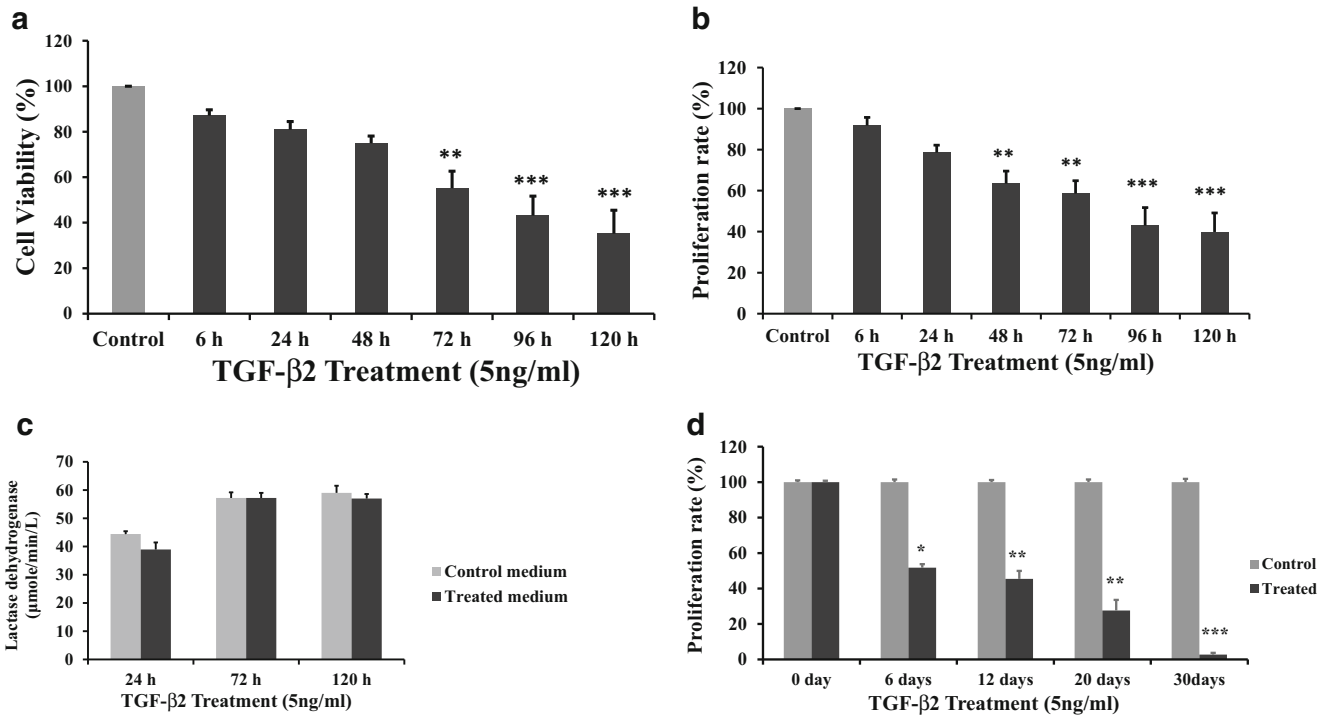


**Fig. 1** Transforming growth factor beta (TGF-β) 2 inhibits cell viability and proliferation of HT-29 human colon adenocarcinoma cells. **a** Effect of TGF-β2 on the viability of HT-29 cells determined by trypan blue exclusion assay. **b** Effect of TGF-β2 on the proliferation of HT-29 cells detected by MTT assay. **c** Cytotoxic effects of TGF-β2 on HT-29 cells was detected by LDH cytotoxicity assay. **d** MTT assay showed maximal inhibitory effect of TGF-β2 on the proliferation potential of HT-29 cells. $n = 6$ experiments were performed. All the data were presented as the mean ± SEM. $^{*}p < 0.05$, $^{**}p < 0.001$ and $^{***}p < 0.001$, compared with untreated control group, one-way ANOVA with the Bonferroni multiple comparison post-test

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## Compliance with ethical standards

**Conflict of interest** The authors declare that they have no conflict of interest.

**Ethical approval** This article does not contain any studies with human participants or animals performed by any of the authors.

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