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Pro-inflammatory cytokine interleukin-1β promotes the development of intestinal stem cells

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Abstract

Objective: We investigated the effect of IL-1β on the development of intestinal epithelial stem cells.

Materials and methods: Normal intestinal epithelial cell line IEC-18 cells were cultured in the presence or absence of 200 pM of IL-1β in serum-free medium (SFM) for various time periods. The effects of IL-1β on intestinal stem cell self-renewal and IEC-18 cell proliferation were evaluated by a colony formation assay, MTT assay, and a focus formation assay. The expression of stemness genes including Bmi-1, Lgr-5, c-myc, Nanog, and β-catenin in IEC-18 cells were measured by quantitative PCR and western blot analysis.

Results: IEC-18 cells grew as a monolayer in SFM in the absence of IL-1β. Cellular spheres were formed when IEC-18 cells were grown in SFM in the presence of IL-1β. IL-1β induced the development of large colonies in the soft-agar as well as the formation of foci when IEC-18 cells were cultured in type-I collagen coated plates. The expression of Bmi-1, Lgr-5, c-myc, Nanog, and β-catenin were significantly increased in IEC-18 cells treated with IL-1β.

Conclusion: Our studies provide direct evidence the IL-1β may play an important role in the self-renewal of intestinal epithelial stem cells and the development of cancer stem cells.

Key Words IL-1β, stem cells, intestinal epithelial, IEC-18, cancer, self-renewal

Introduction

The renewal of intestinal epithelium is a tightly controlled process and essential for maintaining the integrity of the mucosa, repairing mucosal injury, and replenishing the specialized cells of the epithelium. Alterations in epithelial renewal are closely involved in transformation of the epithelium to benign and malignant tumors. It has been suggested that homeostasis of the intestinal epithelium is maintained by an intestinal stem cell (ISC) compartment that resides at the bottom of the crypt of the small and large intestine [1].

The location and behavior of ISCs within the base of intestinal crypt have been characterized by numerous investigators using various animal models [2, 3]. Current literature support two different hypothesis of ISCs: one hypothesis suggests that ISCs are located above the Paneth cells (+4 position), expressing Bmi-1, and normally maintained in a quiescent state through direct interaction with and signals from the niche [2]. The other hypothesis implies that ISCs are crypt base columnar (CBC) cells that are located between the Paneth cells, continuously activated by signals generated from stromal cells at the crypt base, and responsible for most of the regenerative capacity of the intestine under homeostatic conditions [2]. Although significant progress has been made in the last few decades in intestinal stem cell research, the identity of ISCs is still being debated due to the tremendous technical difficulty in isolating and genetically marking ISCs to definitively demonstrate their stemness. Thus far, the molecular mechanisms regulating maintenance of these ISCs and regeneration of intestinal epithelia are not well understood.

In addition to renewal of intestinal epithelium, intestinal stem cells have also been indicated as the cells of origin of intestinal cancers [4]. Dysregulation of stem cell proliferation has been linked to formation and progression of tumors [5]. Recent studies have shown that inflammation can promote tumorigenesis by inducing hyper-proliferation of gastrointestinal stem cells [6-10]. However, the identities of inflammatory factors responsible for the induction of supernumerary intestinal stem cells are still unclear.

Human patients with inflammatory bowel disease (IBD), including both ulcerative colitis and Crohn's disease have a two-to-three fold greater lifetime risk of developing colorectal cancer compared to the general population [11]. IL-1β, a pleiotropic pro-inflammatory cytokine, is significantly up-regulated in IBD patients [12-14], and blocking IL-1β can result in attenuated disease [15]. Furthermore, up-regulation of IL-1β has been closely associated with gastrointestinal tumor initiation and progression [16, 17]. We have previously shown that mice with a higher level of baseline IL-1β in the intestine are more susceptible to dextran sulfate sodium (DSS)-induced colitis [18]. Our recent discovery that IL-1β promotes the development of brain cancer stem cells (CSCs) from differentiated cancer cells [19] prompted us to determine whether IL-1β can induce the development of intestinal stem cells (ISCs) and CSCs from normal intestinal epithelial cell cultures. Here, we report that IL-1β can promote the development of ISCs from normal intestinal epithelial cell culture and IL-1β-induced ISC expansion leads to the loss of cell contact inhibition that is often demonstrated by transformed cells.

Material and methods

Cell line and culture conditions

A rat normal intestinal epithelial cell line, IEC-18, was a kind gift from Dr. Sherry Fleming (Kansas State University, Manhattan, KS, USA). Cells were cultured in serum-free medium (SFM) which consisted of neurobasal-A medium supplemented with B27, GlutaMAX-I supplement, 1% penicillin-streptomycin (Invitrogen, Carlsbad, CA, USA), 50 ng/ml heparin (Sigma-Aldrich, Saint Louis, MO, USA), 20 ng/ml of EGF, and 20 ng/ml bFGF (R&D systems, Minneapolis, MN, USA). To determine the effects of IL-1β on cell growth, 200 pM IL-1β (R&D Systems) was added every other day to serum-free medium.

Self-renewal assay and cell proliferation assay

IEC-18 cells at a clonal density of 1 cell/μl in serum-free medium were seeded at 100 μl/well in 96-well plates and treated with or without 200 pM IL-1β for seven days. IL-1β was added every other day. The total number of cells in each well was counted under a microscope after trypan blue staining. Cell proliferation was measured using the cell proliferation kit I (MTT, Roche Applied Science, Indianapolis, IN, USA) as described by the manufacturer.

Soft agar assay – Colony formation assay

The soft agar assay was performed in six-well plates containing two layers of Sea Plague Agar (Invitrogen). The bottom layer consisted of 0.8% agar in 1 ml of SFM. Single IEC-18 cells ($1x10^5$/well) were placed in the top layer containing 0.4% agar in SFM. The top layer agar was covered with 0.5 ml of SFM with or without 200 pM IL-1β. The top medium was changed every four days and fresh IL-1β was added to the top medium every other day. Cells were cultured for 40 days. Colonies were photographed under a microscope and measured using the ImageJ program (imagej.nih.gov). Colonies with diameters larger than 30μm were counted.

Focus formation assay

24-well plates were coated with Type I collagen (Angiotech BioMaterials Corp., Palo Alto, CA, USA). IEC-18 cells ($3x10^4$/well) were cultured in SFM in the presence or absence of 200 pM IL-1β. Media were changed every four days and fresh IL-1β was added every two days. After two weeks of culture, cells were stained with Giemsa (Thermo Fisher Scientific, Waltham, MA, USA) and the number of foci in each well was counted under a microscope.

RNA extraction and quantitative RT-PCR

Total RNAs were extracted using TRI reagent (Sigma-Aldrich), followed by digestion with a DNase kit (Applied Biosystems, Carlsbad, CA, USA) to remove DNA residues. Reverse transcription was carried out using the iScript cDNA synthesis kit (Bio-Rad, Hercules, CA, USA) and quantitative real-time PCR was performed using SsoFast Eva Green Supermix kit (Bio-Rad).

Western blot analysis

IEC-18 cells were cultured in SFM in the absence or presence of IL-1β for seven days. Cells were then washed with cold PBS, lysed in RIPA buffer [25 mM Tris-HCl (pH 7.6), 150 mM NaCl, 1% NP-40, 1% sodium deoxycholate, 0.1% SDS) and pelleted by centrifugation. Protein concentrations were determined using a NanoDrop instrument (Thermo Fisher Scientific). Cell lysates (30 μg protein for each sample) were incubated for 5 min at 100^0C in 2x loading buffer, separated by electrophoresis in 10% polyacrylamide gels, and transferred to PVDF membranes (Millipore, Bedford, MA, USA). Membranes were blocked with 5% milk in PBS and then incubated with a primary antibody anti-Bmi-1 clone F6 (1:1000 dilution, Millipore), anti-β-catenin (1:1000, Cell Signaling, Boston, MA, USA), or anti-β-actin (1:1000 dilution, Sigma), and a secondary antibody HRP-conjugated goat anti-mouse IgG-HRP (1:1000 dilution, Millipore) or anti-rabbit IgG HRP-linked antibody (1:1000 dilution, Cell Signaling), respectively. Detection was performed using HyGLO substrate (Denville Scientific, Metuchen, NJ, USA) and images were taken using the AlphaEaseFC imaging system (Cell Biosciences, Santa Clara, CA, USA).

Statistical analysis

Student's *t* test was used to determine statistical significance for all analyzed data. A two-sided $p< 0.05$ was considered significant.

Results

IL-1β induces sphere formation and colony formation of IEC-18 cells in serum-free medium

Consistent with previous reports by others [20, 21], we also found that rat intestinal epithelial cell line IEC-18 cells exhibit a number of characteristic features of normal intestinal epithelia cells in culture: strong cell-cell contact or density inhibition of growth, lack of growth in soft agar, and a low plating efficiency when seeded at a low density. To determine whether there are any active stem cells in IEC-18 cells, we cultured these cells in serum-free medium supplemented with bFGF and EGF (SFM). Serum-free medium (SFM) is routinely used to maintain stem cells at an undifferentiated stem cell state, while bFGF and EGF induce proliferation of normal and neoplastic epithelial stem cells as sphere-like cellular aggregates [22-24]. We found that IEC-18 cells proliferated as a monolayer culture in SFM, suggesting that normal IEC-18 cells do not have active stem cells. However, addition of IL-1β to SFM induced some IEC-18 cells to proliferate as spheres (Fig. 1). Meanwhile, we compared the proliferation rates of IEC-18 monolayer cells and IL-1β-induced sphere cells using a MTT assay and trypan-blue staining method. As shown in Fig.1b & 1c, IL-1β-induced sphere cells grew significantly ($p<0.05$) slower than the untreated monolayer cells.

To verify that IL-1β-induced spheres are not the result of cell aggregation, we performed soft agar colony formation assays in serum-free conditions. Under these conditions, cells are separated by semisolid culture medium to prevent aggregation. More importantly, this assay can distinguish between normal differentiated cells and stem cells. Differentiated cells undergo

anoikis in the absence of anchorage to a substratum, while stem cells can survive in anchorage-independent conditions and form colonies [24]. To perform the colony formation assay, IEC-18 cells were placed in soft agar in SFM with or without IL-1β and cultured for 40 days. In the absence of IL-1β, most IEC-18 cells in the soft agar underwent anoikis and no colonies were formed. However, some colonies were generated among the IEC-18 cells treated with IL-1β (Fig. 1d, e). The efficiency of colony forming cells was around 0.03% (Fig. 1f), indicating that IL-1β stimulates a rare sub-population of IEC-18 cells to self-renew, proliferate, and form colonies.

In addition, we also evaluated whether IL-1β-induced sphere IEC-18 cells still maintained self-renewal ability without IL-1β. IL-1β-induced spheres were dissociated and cultured in serum-free medium without IL-1β for seven days. The cytokine-withdrawn cells proliferated as monolayer cells and exhibited similar morphology as control cells without cytokine treatment (data not shown). This result suggests that constant presence of IL-1β in the medium is required for the activation and maintenance of ISC self-renewal.

IL-1β induces loss of contact inhibition of IEC-18 cells in SFM

Contact inhibition is the natural process of arresting cell growth when normal cells contact nearby cells. However, malignant cells lose this property and continue to divide, forming a mass of cells as a tumor. In culture, normal cells grow in a single layer on the substratum but malignant cells continue to grow and form excess layers of cells, called foci. Therefore, we examined whether IL-1β-induced intestinal stem cells possess properties of cancer cells by doing focus assays. To determine whether IL-1β can cause IEC-18 cells to grow without contact

inhibition, plates were coated with collagen to allow cells to grow as adherent cells in SFM with or without IL-1β (Fig. 2a). The effects of IL-1β on IEC-18 cell proliferation were evaluated before cells reached confluency using a MTT assay. As shown in Fig. 2b, IL-1β treated cells proliferated significantly ($p<0.05$) slower than control cells. When cells reached confluency, control cells stopped growing and formed a monolayer culture. However, IL-1β-treated cells continued to grow and formed excess layers of cells (foci).

To confirm focus formation, the above cell cultures were stained with Giemsa on day 14. In this assay, monolayer cells were in grey while foci were in purple (Fig. 2c), indicating piling of cells in foci. The number of purple foci in each well was counted under a microscope. As shown in Fig. 2d, foci were formed only when IEC-18 cells were treated with IL-1β. To further verify the formation of foci, we also compared the total number of IEC-18 cells in each well treated with or without IL-1β for 14 days after they reached confluency. As shown in Fig. 2e, there were significantly more IEC-18 cells in the wells with IL-1β than that in the wells without IL-1β. This result is consistent with the microscopic observation that multilayer of cells (focus formation) present in IL-1β-treated wells.

IL-1β induces expression of stem cell markers in IEC-18 cells in SFM

To determine the molecular mechanisms of IL-1β-induced colony and focus formation, we compared the expression of several stemness genes in IEC-18 cells treated with or without IL-1β in SFM. As shown in Fig. 3a, the expression of stem cell markers Bmi-1, Lgr5, c-myc, β-catenin and Nanog were significantly ($p<0.01$) increased in IL-1β-treated cells cultured in SFM,

compared to that in control cells cultured in the same condition. IL-1β-induced protein expression of Bmi-1 and β-catenin was further confirmed by western blot analysis (Fig. 3b). These results suggest that IL-1β induces stemness gene expression, leading to colony and focus formation in SFM.

Discussion

The homeostasis of intestinal epithelium renewal is essential for maintaining the structural and functional integrity of intestinal mucosa. Dysregulation of the self-renewal of intestinal epithelium may lead to the development of gastrointestinal cancers. Therefore, it is not surprising that the host has developed a tightly controlled system to maintain efficient interaction between intestinal stem cells (ISCs), their progenies, and the microenvironment. Although a significant amount of literature support the notion that ISCs can be the cells of origin for intestinal cancers [4] and inflammation may play an important role in ISC-mediated tumorigenesis [6-10], how ISC renewal and intestinal cancer stem cells are regulated by inflammatory cytokines remains obscure. Here, we present data that IL-1β can promote self-renewal and proliferation of intestinal epithelial stem cells and regulate the development of intestinal cancer stem cells.

IEC-18 cells are normal small intestinal crypt cells that were established in vitro from the ileum of outbred germfree Crl:CD(SD)GN rats [20]. Because IEC-18 cells in serum-free medium demonstrate a monolayer culture and lack of growth in soft agar, we speculate that normal IEC-18 cells do not contain intestinal stem cells (ISCs) or the ISCs are inactive.

Interestingly, addition of IL-1β to the cell culture caused monolayer cells to form spheres expressing up-regulated "stemness" genes including Lgr-5, Bmi-1, cMyc-1, β-catenin, and Nanog. The sphere cells proliferate at a slower rate compared with control monolayer IEC-18 cell, and this is consistent with the observation that stem cells grow slower than differentiated cells [25]. Because only a small portion (0.03%) of IEC-18 cells possess stem cell properties, it is likely that normal IEC-18 cell cultures contain a rare population of quiescent intestinal stem cells that can be reactivated by IL-1β. However, our studies cannot rule out the possibility that these colonies and spheres are the *de novo* induction of stem cells from normal progenitor cells by IL-1β. Nonetheless, it is reasonable to conclude that IL-1β can support the development of intestinal epithelial stem cells.

Bmi-1, a transcriptional repressor belonging to the polycomb group protein family, is a well-recognized molecular marker for ISCs. The leucine-rich, repeat-containing G protein-coupled receptor (Lgr) 5, also called GPR49, is another marker of stem cells in adult intestinal epithelium [3]. Although the exact function of Lgr-5 is unknown, it represents a different group of stem cells in the intestine because Lgr-5 is mainly expressed in the crypt base columnar cells which are located between the Paneth cells, while Bmi-1 is expressed in +4 cells which are located above the Paneth cells [2]. Thus, these two markers label two different states of ISCs. Lgr-5-expressing cells are actively proliferating stem cells responsible for the daily maintenance of the intestine epithelium, while Bmi-1-expressing cells are normally maintained in a quiescent state [2]. Recent studies on these two stem cell populations indicate that Bmi-1-expressing cells are up-stream of the rapidly dividing Lgr-5^{+} cells and replenish the pool of active stem cells under normal circumstances [26]. Moreover, Bmi-1-expressing cells can also directly give rise to all

intestinal cell types without transition to Lgr-5^+ cells in Lgr-5-deficient mice, indicating that Lgr-5 expression is dispensable for the homeostasis of intestinal epithelium [26]. However, ablation of Bmi-1-expressing cells can lead to depletion of whole crypt units [27]. Thus, Bmi-1-expressing cells appear to be more critical than Lgr-5^+ cells for crypt maintenance. Our studies have shown that the expression of Bmi-1 and Lgr-5 is significantly enhanced in IL-1β –induced IEC-18 sphere cells. The finding suggests that there were two subsets of ISCs present in the original IEC-18 cell line when they were isolated from rat intestinal crypt, and IL-1β promotes the expansion of both stem cell populations. Further studies are needed in order to determine whether Lgr-5^+ IEC-18 cells are derived from the Bmi-1-expressing IEC-18 cells.

It has been suggested that IL-1β alone can induce inflammation and gastric cancer through recruitment and activation of myeloid-derived suppressor cells (MDSCs) [16]. MDSCs serve as a source for IL-6 production and IL-6-induced activation of STAT3 in epithelial cells can lead to tumor initiation [16, 28, 29]. Different from the previous report, we have shown that IL-1β could act directly on IEC-18 cells to promote the expression of both Bmi-1 and Lgr-5 and a functional phenotype of loss of contact inhibition of growth and anchor-independent colony formation, typical characteristics of cancer cells. Results from our studies are consistent with the notion that Bmi-1 and Lgr-5 not only are hall markers of intestinal stem cells, but also are strongly expressed in gastrointestinal neoplasias [2, 3]. Thus, our data suggest that IL-1β could directly target epithelial cells to induce ISC-mediated tumorigenesis.

Canonical Wnt/β-catenin pathway is a key signaling mechanism for intestinal proliferation, maintenance of ISCs, and development of colorectal cancer cells. Wnt signaling regulates β-

catenin's stability, accumulation in the cytoplasm, and translocation into the nucleus [30]. Both intestinal stem cell markers Bmi-1 and Lgr-5 have been shown to be the downstream targets of Wnt/β-catenin signaling [31, 32]. Because IL-1β can significantly enhance the expression of β-catenin, Bmi-1 and Lgr-5 in IEC-18 cells (Fig. 3), it is possible that the effect of IL-1β on ISC self-renewal and cancer stem cell development is mediated by the Wnt/β-catenin pathway. This notion is consistent with the report that IL-1β alone is sufficient to activate Wnt signaling and is required for increased Wnt signaling in colon cancer cells [33]. However, further studies are required to determine the molecular mechanism of IL-1β-mediated Wnt/β-catenin signaling in intestinal stem cells and cancer stem cells.

Numerous studies have demonstrated that the development and maintenance of intestinal stem cells and cancer cells are regulated by inflammation in the intestinal crypt. Therefore, it is reasonable to speculate that key inflammatory cytokines such as IL-1β may be involved in the micro-environmental regulation of stem cells in intestinal homeostasis and cancer. Under physiological conditions, it is likely that inflammation-responsive ISCs would become quiescent again after tissue wound is healed and inflammation is resolved. However, persistently over-expression of IL-1β in chronic inflammation could induce over-proliferation of ISCs. Our studies provide the first evidence that IL-1β can directly act on intestinal epithelial cells to activate ISC self-renewal, which in turn contributes to inflammation-mediated epithelial repair and/or tumorigenesis depending on the intensity and duration of intestinal inflammation.

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Figure legends

Fig. 1 IL-1β induced sphere and colony formation of IEC-18 cells. **a** IL-1β induced sphere formation of IEC-18 cells in SFM. IEC-18 cells ($3x10^4$/well) were cultured in 24-well plates containing SFM in the presence or absence of IL-1β for 7 days. Scale bar = 400 μm. **b & c** IL-1β inhibited proliferation of IEC-18 cells in SFM. IEC-18 cells ($2x10^4$/well) were cultured in 96-well plates containing SFM with or without IL-1β for 7 days. Cell proliferation was determined using a MTT assay (**b**) or cell count under a microscope after cells were dissociated and stained with Trypan blue (**c**). Error bars represent SEM. *$p < 0.002$. **d**. IL-1β induced colony formation of IEC-18 cells in soft agar containing SFM. IEC-18 cells ($1x10^5$/well) were cultured in 6-well plates containing soft agar in SFM with or without IL-1β for 40 days. Scale bar = 200 μm. (**e**). Number of colonies (>30 μm) that were measured and counted under a microscope. (**f**) Percentages of colony-forming cells calculated using the number of colonies divided by the number of seeded cells. Error bars represent SEM. *$p < 0.001$.

Fig. 2 IL-1β induced focus formation of IEC-18 cells in SFM. **a** Representative images of IEC-18 cells cultured in collagen-coated 6-well plates containing SFM with or without IL-1β for 2 days. Scale bar = 200 μm. **b** IL-1β inhibited the proliferation of adherent IEC-18 cells in SFM

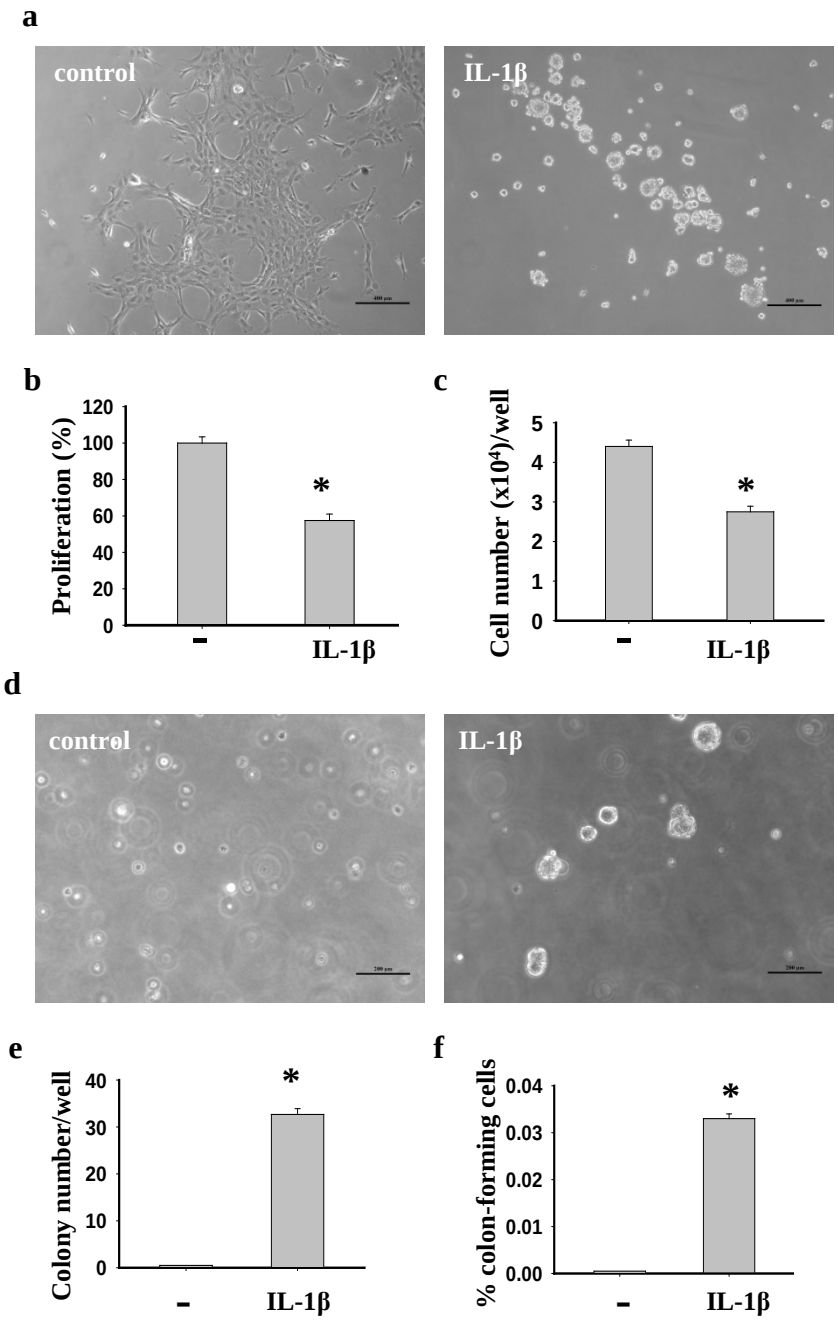
before cells reached confluency. IEC-18 cells ($3x10^3$/well) were cultured in collagen-coated 96-well plates containing SFM with or without IL-1β for 8 days. Cell proliferation was determined using a MTT assay. OD values represent the amount of viable cells at each time point. *$p < 0.02$. **c** Representative images of Giemsa-stained IEC-18 cells treated with or without IL-1β for 14 days. IEC-18 cells (3×10^4/well) were cultured in collagen-coated 24-well plates containing SFM with or without IL-1β for 14 days. Cells were stained with Giemsa. Scale bar = 300 μm. **d** The number of foci counted in a microscopic field. Error bars represent SEM. *$p < 0.001$. **e** IL-1β induced proliferation of IEC-18 cells in SFM after cells reached confluency. IEC-18 cells ($3x10^5$/well) were cultured in collagen-coated 24-well plates containing SFM with or without IL-1β for 14 days. Then cells were dissociated, stained with Trypan blue and counted under a microscope. Error bars represent SEM. *$p < 0.03$.

Fig. 3 IL-1β induced expression of stem cell markers in IEC-18 cells in SFM. **a** Transcriptional levels of stem cell markers in control and IL-1β-treated IEC-18 cells. IEC-18 cells ($5x10^5$/well) were cultured in 6-well plates containing SFM with or without IL-1β for seven days. Differential mRNA levels of stem cell markers were determined by qRT-PCR. β-actin was used as an internal normalization control. Error bars represent SEM. *$p < 0.01$. **b** Immunoblot analysis of Bmi-1 and β-catenin on cell lysates from control cells and IL-1β-treated cells. β-actin was used as an internal normalization control.

References

1. Medema JP, Vermeulen L. Microenvironmental regulation of stem cells in intestinal homeostasis and cancer. Nature. 2011;474(7351):318-326.
2. Umar S. Intestinal stem cells. Current gastroenterology reports. 2010;12(5):340-348.
3. Barker N, Clevers H. Tracking down the stem cells of the intestine: strategies to identify adult stem cells. Gastroenterology. 2007;133(6):1755-1760.
4. Barker N, Ridgway RA, van Es JH et al. Crypt stem cells as the cells-of-origin of intestinal cancer. Nature. 2009;457(7229):608-611.
5. Radtke F, Clevers H. Self-renewal and cancer of the gut: two sides of a coin. Science (New York, N.Y. 2005;307(5717):1904-1909.
6. Jiang H, Patel PH, Kohlmaier A et al. Cytokine/Jak/Stat signaling mediates regeneration and homeostasis in the Drosophila midgut. Cell. 2009;137(7):1343-1355.
7. Cronin SJ, Nehme NT, Limmer S et al. Genome-wide RNAi screen identifies genes involved in intestinal pathogenic bacterial infection. Science (New York, N.Y. 2009;325(5938):340-343.
8. Amcheslavsky A, Jiang J, Ip YT. Tissue damage-induced intestinal stem cell division in Drosophila. Cell Stem Cell. 2009;4(1):49-61.
9. Buchon N, Broderick NA, Poidevin M et al. Drosophila intestinal response to bacterial infection: activation of host defense and stem cell proliferation. Cell Host Microbe. 2009;5(2):200-211.
10. Apidianakis Y, Pitsouli C, Perrimon N et al. Synergy between bacterial infection and genetic predisposition in intestinal dysplasia. Proceedings of the National Academy of Sciences of the United States of America. 2009;106(49):20883-20888.
11. Bernstein CN, Blanchard JF, Kliewer E et al. Cancer risk in patients with inflammatory bowel disease: a population-based study. Cancer. 2001;91(4):854-862.
12. El-Omar EM, Carrington M, Chow WH et al. The role of interleukin-1 polymorphisms in the pathogenesis of gastric cancer. Nature. 2001;412(6842):99.
13. Cominelli F, Pizarro TT. Interleukin-1 and interleukin-1 receptor antagonist in inflammatory bowel disease. Aliment Pharmacol Ther. 1996;10 Suppl 2(49-53; discussion 54.
14. Siegmund B. Interleukin-1beta converting enzyme (caspase-1) in intestinal inflammation. Biochemical pharmacology. 2002;64(1):1-8.
15. Hamilton MJ, Snapper SB, Blumberg RS. Update on biologic pathways in inflammatory bowel disease and their therapeutic relevance. Journal of gastroenterology. 2012;47(1):1-8.
16. Tu S, Bhagat G, Cui G et al. Overexpression of interleukin-1beta induces gastric inflammation and cancer and mobilizes myeloid-derived suppressor cells in mice. Cancer cell. 2008;14(5):408-419.
17. Miki C, Konishi N, Ojima E et al. C-reactive protein as a prognostic variable that reflects uncontrolled up-regulation of the IL-1-IL-6 network system in colorectal carcinoma. Digestive diseases and sciences. 2004;49(6):970-976.
18. Shi J, Aono S, Lu W et al. A novel role for defensins in intestinal homeostasis: regulation of IL-1beta secretion. J Immunol. 2007;179(2):1245-1253.
19. Wang L, Liu Z, Balivada S et al. Interleukin-1beta and transforming growth factor-beat cooperate to induce neurosphere formation and increase tumorigenicity of adherent LN-229 glioma cells. Stem Cell Research and Therapy. 2012;(in press)(
20. Quaroni A, Isselbacher KJ. Cytotoxic effects and metabolism of benzo[a]pyrene and 7,12-dimethylbenz[a]anthracene in duodenal and ileal epithelial cell cultures. Journal of the National Cancer Institute. 1981;67(6):1353-1362.
21. Quaroni A, Isselbacher KJ, Ruoslahti E. Fibronectin synthesis by epithelial crypt cells of rat small intestine. Proceedings of the National Academy of Sciences of the United States of America. 1978;75(11):5548-5552.

22. Ricci-Vitiani L, Lombardi DG, Pilozzi E et al. Identification and expansion of human colon-cancer-initiating cells. Nature. 2007;445(7123):111-115.
23. Dontu G, Abdallah WM, Foley JM et al. In vitro propagation and transcriptional profiling of human mammary stem/progenitor cells. Genes Dev. 2003;17(10):1253-1270.
24. Dontu G, Wicha MS. Survival of mammary stem cells in suspension culture: implications for stem cell biology and neoplasia. J Mammary Gland Biol Neoplasia. 2005;10(1):75-86.
25. Tavakoli T, Xu X, Derby E et al. Self-renewal and differentiation capabilities are variable between human embryonic stem cell lines I3, I6 and BG01V. BMC cell biology. 2009;10(44.
26. Tian H, Biehs B, Warming S et al. A reserve stem cell population in small intestine renders Lgr5-positive cells dispensable. Nature. 2011;478(7368):255-259.
27. Sangiorgi E, Capecchi MR. Bmi1 is expressed in vivo in intestinal stem cells. Nature genetics. 2008;40(7):915-920.
28. Bollrath J, Phesse TJ, von Burstin VA et al. gp130-mediated Stat3 activation in enterocytes regulates cell survival and cell-cycle progression during colitis-associated tumorigenesis. Cancer Cell. 2009;15(2):91-102.
29. Grivennikov S, Karin E, Terzic J et al. IL-6 and Stat3 are required for survival of intestinal epithelial cells and development of colitis-associated cancer. Cancer Cell. 2009;15(2):103-113.
30. Moon RT, Bowerman B, Boutros M et al. The promise and perils of Wnt signaling through beta-catenin. Science. 2002;296(5573):1644-1646.
31. Haegebarth A, Clevers H. Wnt signaling, lgr5, and stem cells in the intestine and skin. The American journal of pathology. 2009;174(3):715-721.
32. Yu T, Chen X, Zhang W et al. Regulation of the potential marker for intestinal cells, Bmi1, by beta-catenin and the zinc finger protein KLF4: implications for colon cancer. The Journal of biological chemistry. 2012;287(6):3760-3768.
33. Kaler P, Augenlicht L, Klampfer L. Macrophage-derived IL-1beta stimulates Wnt signaling and growth of colon cancer cells: a crosstalk interrupted by vitamin D3. Oncogene. 2009;28(44):3892-3902.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IL-1β

Figure 1

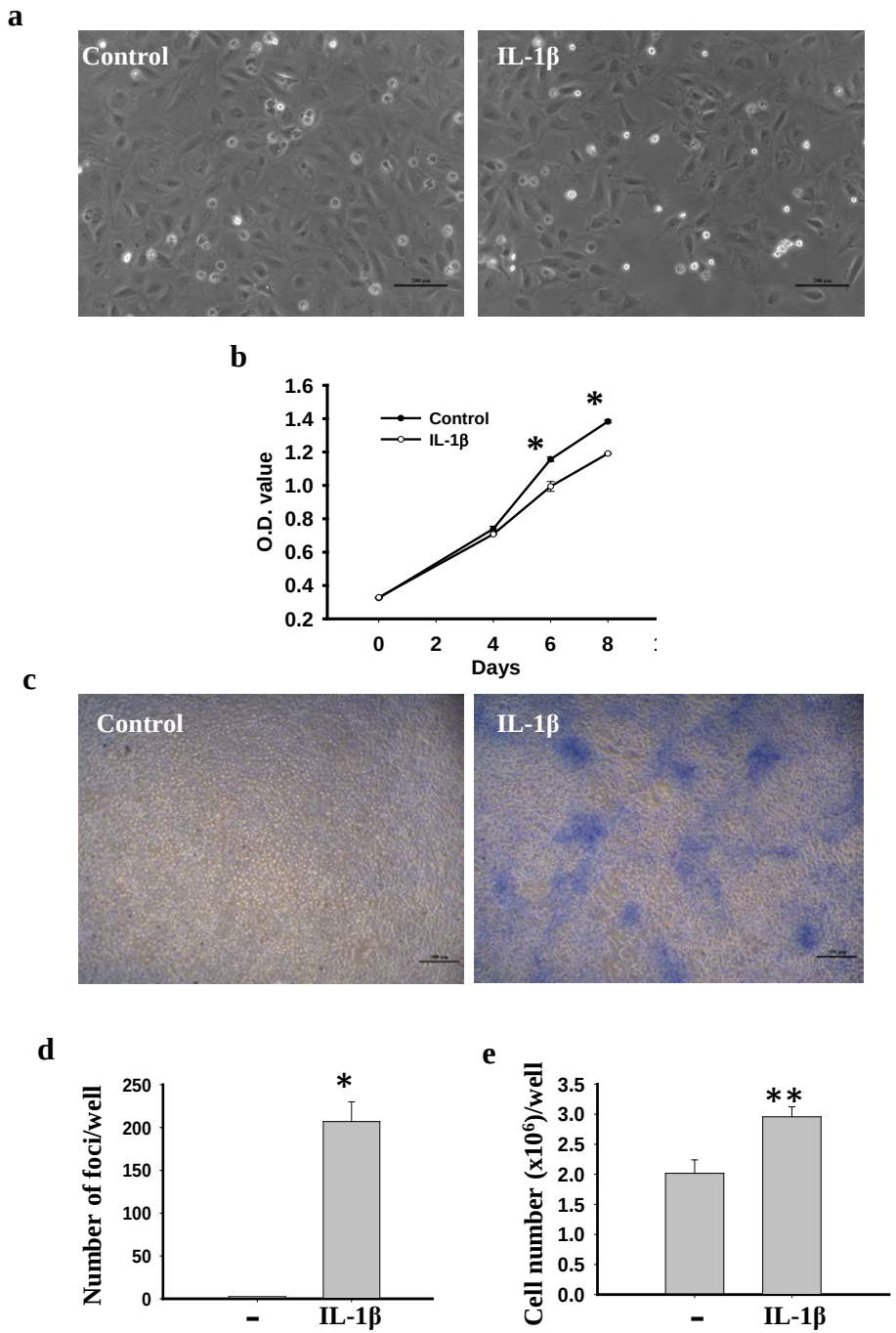
a
Control
IL-1β
b
O.D. value
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
Control
IL-1β
*
*
0
2
4
6
8
Days
c
Control
IL-1β
d
Number of foci/well
250
200
150
100
50
0
*
–
IL-1β
e
Cell number (x10⁶)/well
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
**
–
IL-1β

Figure 2

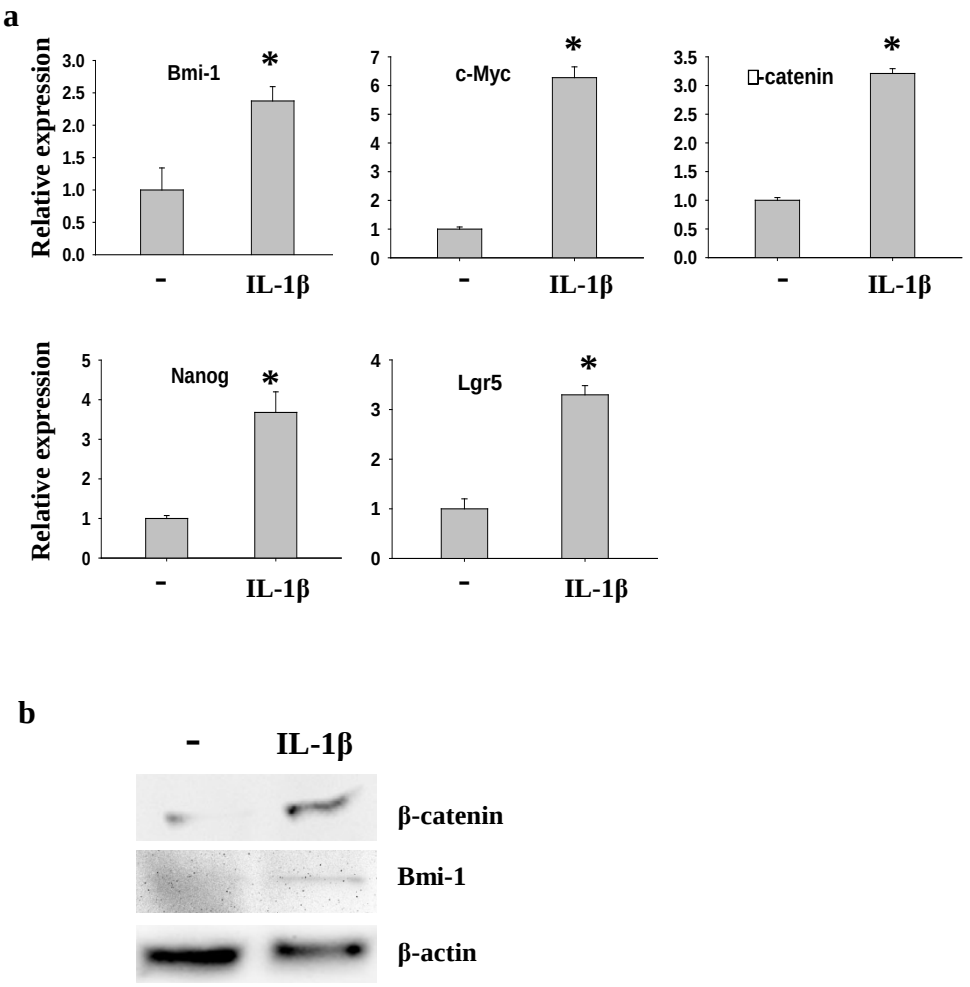
a
Relative expression
Bmi-1
*
3.0
2.5
2.0
1.5
1.0
0.5
0.0
-
IL-1β
c-Myc
*
7
6
5
4
3
2
1
0
-
IL-1β
□-catenin
*
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
-
IL-1β
Relative expression
Nanog
*
5
4
3
2
1
0
-
IL-1β
Lgr5
*
4
3
2
1
0
-
IL-1β
b
-
IL-1β
β-catenin
Bmi-1
β-actin

Figure 3