RESEARCH

23(97), May - June, 2019

ISSN
2321–7359
EISSN
2321–7367

Medical Science

IL-17 and IL-22 in colitis associated colorectal cancer in mice

Elif Saritas Yuksel[1], Firdevs Topal[1], Özlem Gür[2], Fatih Topal[1], Cengiz Karahanlı[1], Berna Uran[2]

Evidence from animal models suggests that innate lymphoid cells (ILC) and especially IL23 responsive ILC3 may contribute to intestinal carcinogenesis. IL-17 and IL-22 are two major cytokins released from ILC. We have investigated mechanisms responsible for tumour-causing inflammation in a mouse model, which, like human colorectal cancer, exhibits upregulation of IL-22 and IL-17. Our results suggest that the innate immune system participates to gut inflammation and colorectal cancer, suggesting that inflammatory responses can be common to both pathologies and contribute to the development of chronic inflammation associated cancer.

INTRODUCTION

Approximately 2% of colorectal cancer (CRC) is linked to pre-existing inflammation known as colitis-associated cancer (CAC). The risk of developing colorectal cancer increases in chronic gut inflammation such as in inflammatory bowel disease (IBD). The balance between the proinflammatory and anti-inflammatory cytokines affecting epithelial cells with genetic predisposition and environmental trigger causes inflammation-driven colon carcinogenesis.

We have investigated mechanisms responsible for tumour-causing inflammation in a mouse model, which, like human colorectal cancer, exhibits upregulation of IL-22 and IL-17. IL22 has been shown to activate MAPK pathways and promote cancer cell survival. IL22 also has antiapoptotic action through AKT phosphorylation. IL22 increases proinflammatory cytokine production and matrix-degrading molecules in IBD (Andoh, 2005).

Cancer and chronic inflammation share common features and often use shared molecular pathways. The IL-23 axis is a key pathway for intestinal inflammation in many mouse models (Hue, 2006), and its components IL-23R and Stat3 are not only associated with IBD susceptibility (Barrett, 2008) but also involved in inflammation-associated cancer in the skin and colon (Kortylewski, 2009). Il-23 induces Th17-type cells to secrete the cytokines IL-17 and IL-22 (Liang, 2006).

Th17 responses and the action of IL-17 itself were initially thought to be proangiogenic, proinvasive, and tumor growth promoting (Numasaki, 2003). In contrast, other studies described a host-protective role in tumor surveillance, inhibition of proliferation, and inhibition of metastasis (Muranski, 2008 #10). On the other hand, Th17 cells and increased IL-17 seem to be associated with a poor prognosis in CRC (Tosolini, 2011; Wu, 2009).

IL-22 has two opposite functions in the intestinal inflammatory response. On the other hand, it has well described protective functions through induction of epithelial regeneration and the production of antimicrobial peptides (Witte, 2010). IL-22 has also been shown to promote colitis in some settings (Kamanaka, 2011; Geremia, 2011; Grivennikov, 2009). A procarcinogenic role for IL-22 via Stat3 activation was previously shown in several extracolonic cancers (Jiang, 2011). Polymorphisms in IL-22 seem to be associated with a 1.46-fold increased risk for CRC (Thompson, 2010).

One of the ILC subsets, ILC3 secretes IL17 and IL22 depending on the expression of RORγt. IL23 driven IL22 producing ILC have been shown to cause colitis-associated cancer in mice. ILC may play an important amplifying role in IBD and IBD-associated cancer, through secretion of inflammatory cytokines and interaction with other immune and non-immune cells.

Innate lymphoid cells are closely connected to adaptive immune systems, sense environmental changes, and ILC react to microbial and inflammatory changes with cytokine production, limiting pathogen spread, and tissue injury. They react to environmental offensive signals. Newer studies show that ILC are also involved in the pathogenesis of chronic inflammatory disorders, including allergic reactions, inflammatory bowel disease (IBD) and cancer (Artis, 2015).

Innate lymphoid cells (ILC) belong to a family of innate immune cells that share similarities with the phenotype and functions of T lymphocytes (Spits, 2013). Evidence from animal models suggests that ILC and especially IL23 responsive ILC3 may contribute to intestinal carcinogenesis. IL23 driven IL22 producing ILC play an important role in colitis-associated cancer model in T and B cell deficient mice and transgenic expression of IL23 was shown to drive the *de novo* development of duodenal adenomatous tumors that was dependent on IL17 secreting ILC (Kirchberger, 2013; Chan, 2014). PSC–IBD patients colon tissue contain over expression of ILC that may play a role in intestinal inflammation and increased cancer risk in PSC and IBD (Gwela, 2017).

[1]Gastroenterology Department, İzmir Katip Çelebi University, Turkey; [2]Surgery Department, İzmir Katip Çelebi University, Turkey

© 2019 Discovery Publication. All Rights Reserved. www.discoveryjournals.org | OPEN ACCESS

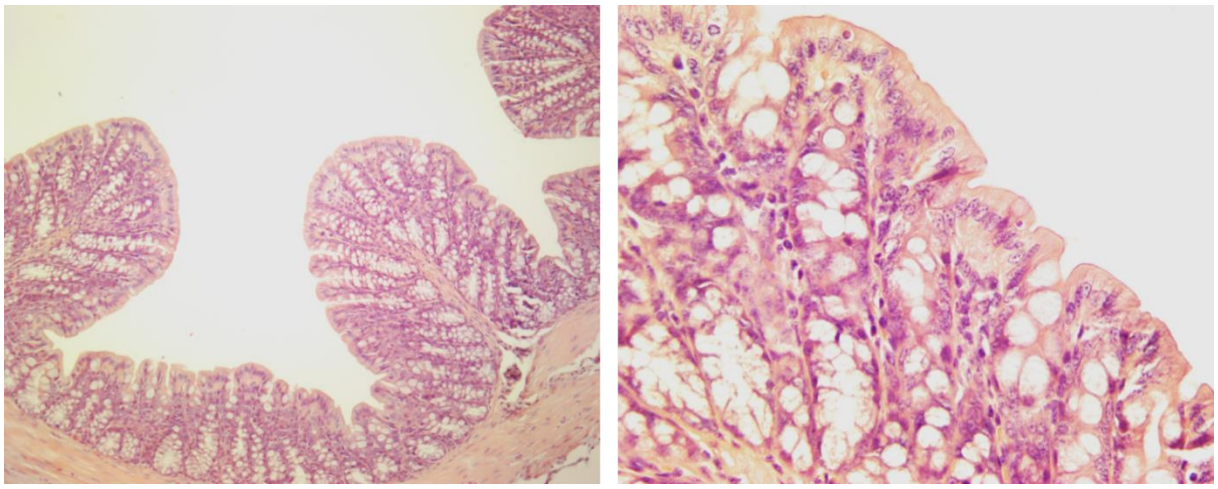


Figure 1 Hematoxilen eosin staining of the mucosa of the control group

We aimed to show the increased expression of IL-17 and IL-22 primarily secreted from ILC in colitis and colitis associated colorectal cancer.

MATERIAL METHOD

The animal experiment was approved by the Institutional Local Animal Care and Use Committee.

The inclusion criteria for the mouse were: Female C57BL/6 mice (C57BL/6J, Kobay Laboratory, Ankara, Turkey), 8–12 weeks old. While mice from both sexes are susceptible to AOM/DSS-induced CAC, based on some author's observations, female mice tend to yield more consistent and reproducible results. All animal experiments were done in accordance with institutional and national guidelines and regulations.

The inflammation-induced colon cancer mouse model as introduced by Tanaka et al. was adapted to the mouse strains used in this study (Tanaka, 2003). There were three groups: control group, colitis group and cancer group. The groups were age and gender-matched with control mice. 8–12 week old C57BL/6 mice were used.

Colitis was induced by administering 4 repeated cycles of DSS, each cycle involving administration of 3% DSS concentrations for 5 days followed by sterile water for 14 days.

Accurate weights were determined in order to ensure uniform dosing ofazometaxan (AOM). The volume of AOM (1 mg/ml) was calculated to inject to achieve a dose of 12 µl /kg. Mice were anesthetized using isoflurane. Each mouse was injected intraperitoneally with the appropriate volume of AOM using a 281/2 G tuberculin syringe. We weighed and monitored them over the next 48 hours. In the third day, we replaced 3% (dextran sodium sulfate) DSS with sterile drinking water for 5 days. 3% DSS was replaced with sterile drinking water for fifteen days recovery period. Two more DSS cylces were produced. In the sixty fifth day (at the end of the third recovery period) the mice were sacrificed.

The mice were euthanized by a combination of inhalational isoflurane overdose and cervical dislocation. Because DSS-induced colitis damages the distal colon, it was critical to remove the entire colon to accurately assess tumor burden. A Swiss roll was formed and the distal colon was in the center and the proximal colon was in the the outermost layer. The rolls were immersed into 10% buffered formalin for 24 hours.

The samples were processed by histological analysis. Hematoxylin and eosin (H&E) staining was performed to assess crypt and tumor pathology. H&E staining of a well-aligned colon rolls allows microscopic examination of tumors and their location within the colon. The severity of inflammatory injury was observed with identification of inflammatory infiltrates and presence of crypt abscess. All features of dysplasia (hyperchromatic nuclei, increased nuclei:cytoplasmic ratio, and eccentric nuclei placement) were searched.

The materials were processed with immunohistochemical staining for IL17 and 22. The tissue samples were embedded in paraffin. Paraffin samples were cut into 5 µm sections and stained with hematoxylin and eosin. Detection of IL-17 and 22 in the tissue was performed with an IL-17 Polyclonal Antibody (Mouse, Rat, Host Rabbitbs-1183R Bioss-USA) and an IL-22 Polyclonal Antibody (Human, Mouse, Rat, Host Rabbitbs-2624R Bioss-USA). Photomicrographs were obtained using an Olympus BX41 microscope, and the average density of the labelling signal was measured using an image analyser.

RESULTS

Each group involved 10 mice. In the control group there were no changes in the colonic gland tissues with the Hemotoxilen eosin staining and there were no immunologic cell infiltration and immunohistochemical staining.

In the colitis group, there were local mononuclear infiltration, edema and perivascular mononuclear migration. We found increased levels of IL-22, IL-17 in the inflamed tissue.

In the CAC model, irregularities in the epithelium, cellular migration to the muscle layer, perivascular mononuclear infiltration. There were nuclear and cytoplasmic abnormalities. High nuclear-cytoplasmic ratios, crowding and pleomorphism were detected. There was a reduction in goblet cell mucin, similar to immature basal crypt epithelium. In DSS-AOM-treated mice, we found increased levels of IL-22, IL-17 in precarcinogenic aberrant crypt foci (ACF) compared with adjacent control tissue.

Statistical Analysis

The statistical analysis showed that the percentage of IL-17 and IL-22 stained cells was significantly higher in CAC compared to the control group. (IL stained cells: 3.28 ± 0.10% vs. 2.04 ± 0.08%, $p < 0.01$). There

© 2019 Discovery Publication. All Rights Reserved. www.discoveryjournals.org | OPEN ACCESS

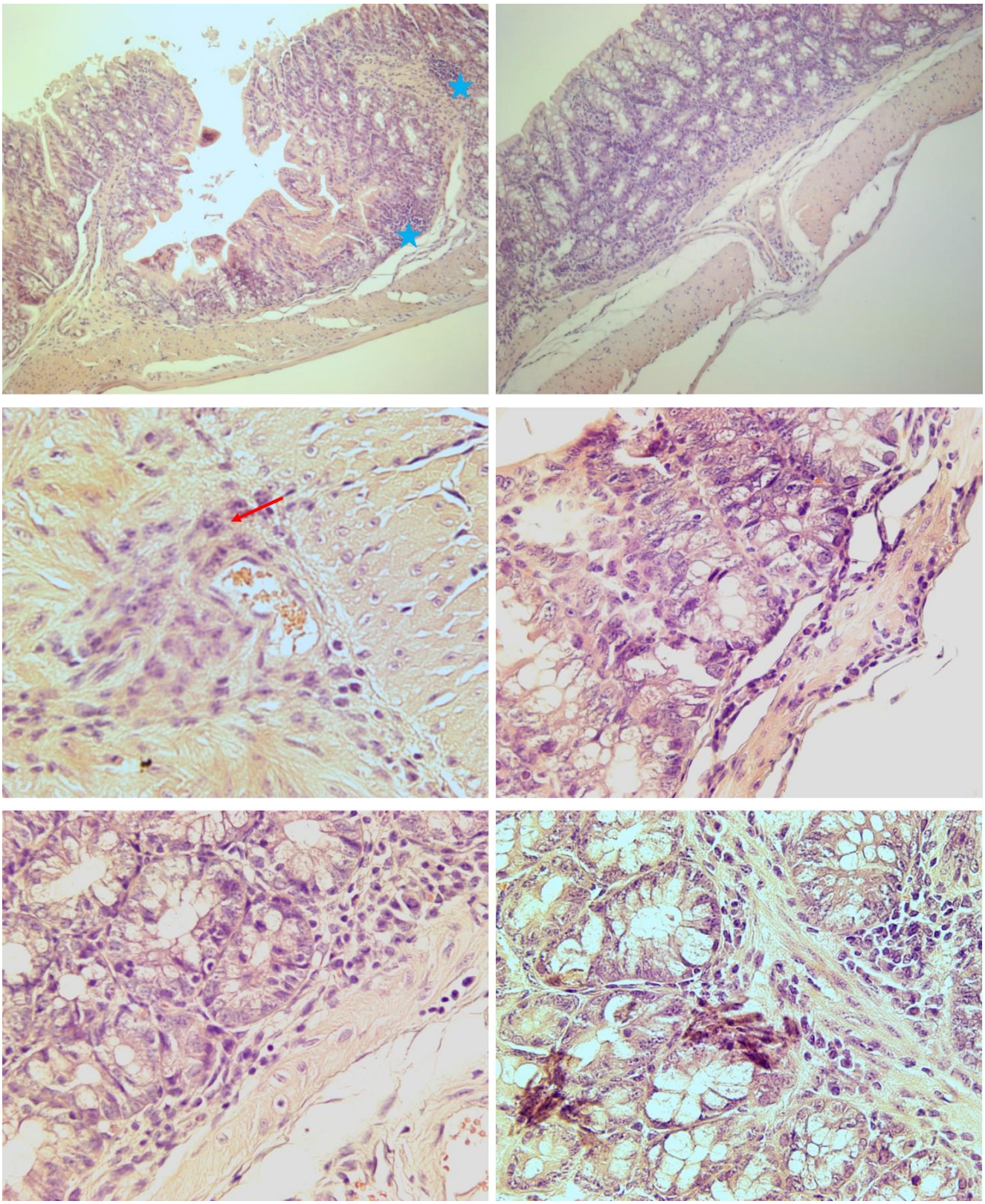


Figure 2 Hematoxilen eosin staining of the colitis group
Blue star: Mononuclear cell infiltration
Red arrow: Perivascular mononuclear migration

© 2019 Discovery Publication. All Rights Reserved. www.discoveryjournals.org | OPEN ACCESS

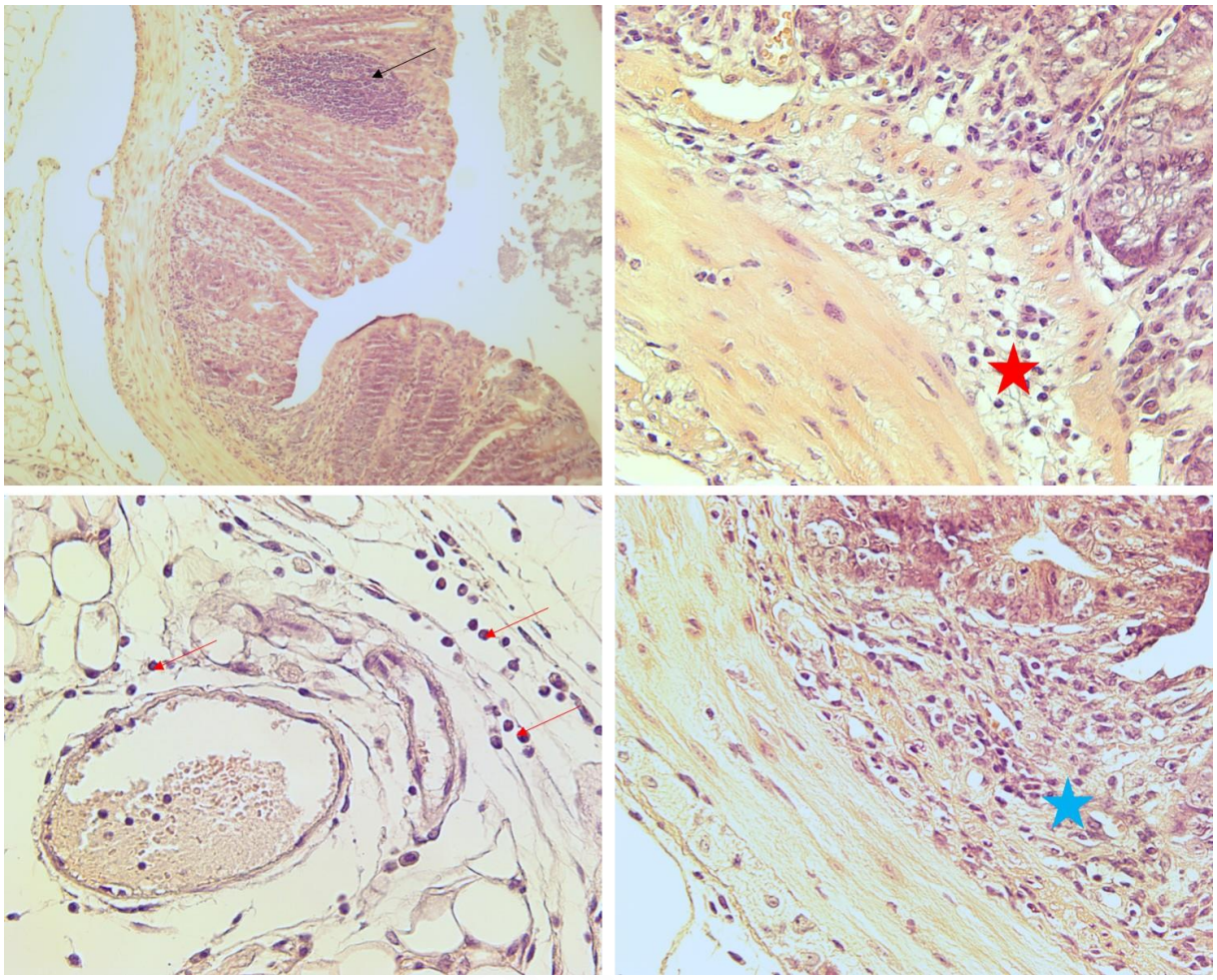
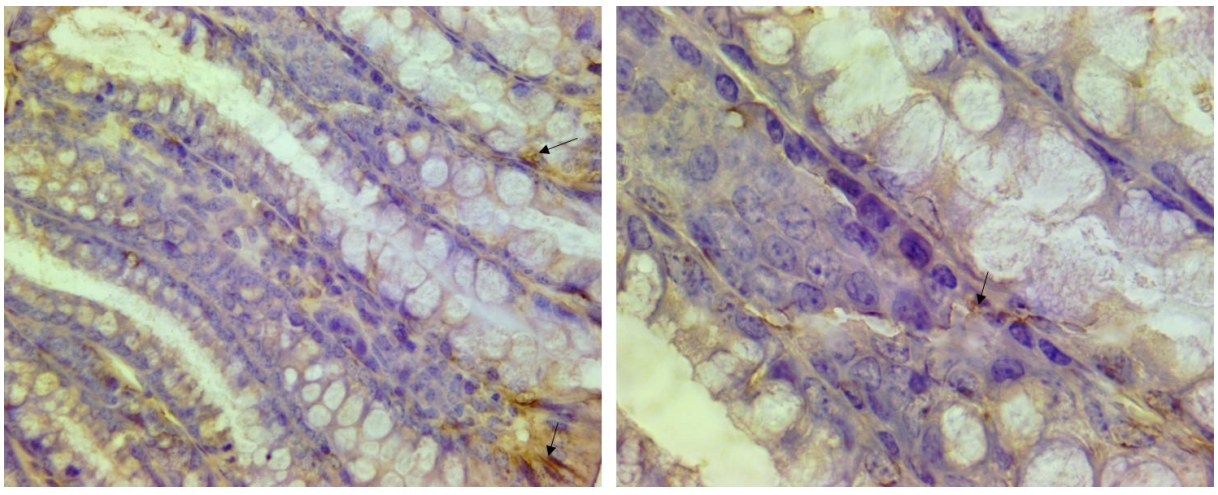


Figure 3 Hematoxilen eosin staining of the CAC group
Red star: Edema and mononuclear cell infiltration
Blue star: Mononuclear cell infiltration
Red arrow: Perivascular mononuclear migration
Black arrow: Lymphositosis



A **B**

Figure 4 IL-17 immunohistochemical staining of the colitis (A) and CAC group(B)
Black arrow: Lymphositosis

© 2019 Discovery Publication. All Rights Reserved. www.discoveryjournals.org | OPEN ACCESS

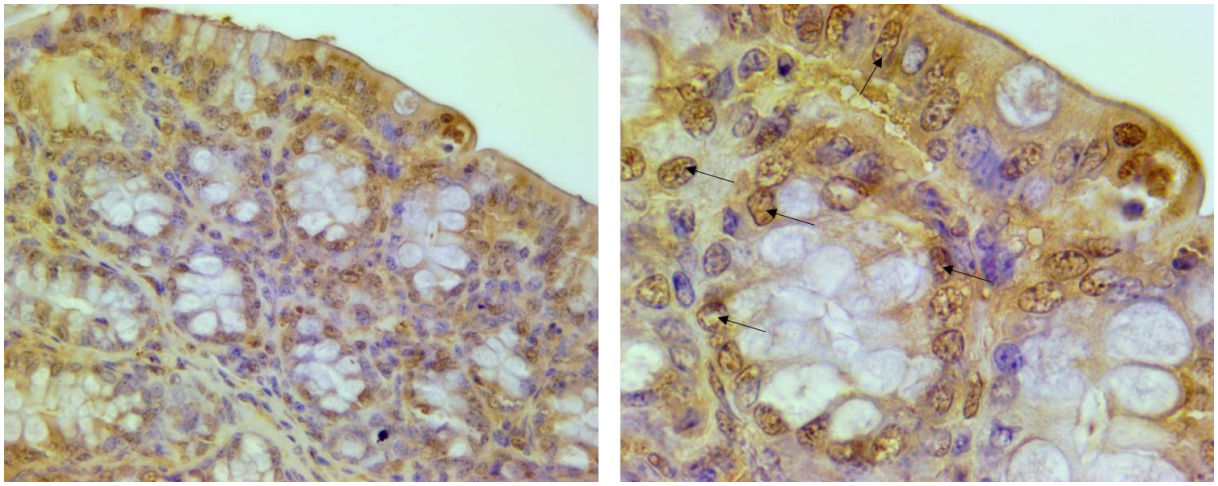


Figure 5 IL-22R staining of the colitis group
Black arrow: Lymphositosis

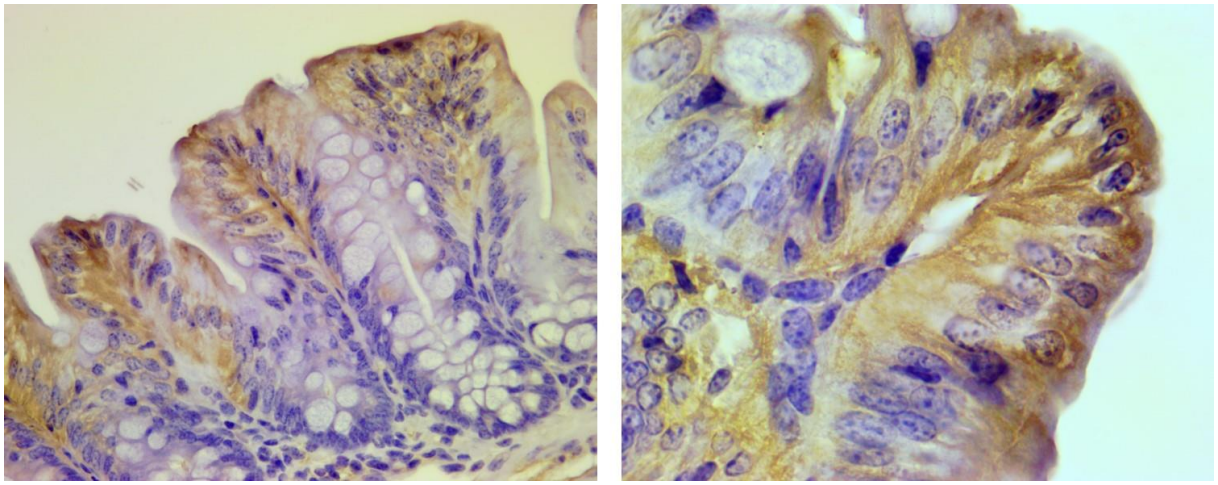


Figure 6 IL-22R staining of the CAC group

were no statistical difference between the colitis group and the CAC group.

DISCUSSION

CAC is an inflammation related cancer model and is very well known and the IBD patients are screened for colon cancer after 8-10 years of disease course. However the risk differs in patients. Therefore identifying the molecular pathways responsible for carcinogenesis would identify which patients to screen vigoriously and use targeted treatment towards the identified risky molecules.

Elevated IL-17 and IL-22 expression has been demonstrated in several human tumors, such as ovarian, prostate, breast, hepatocellular, esophageal, gastric, and non-melanoma skin cancers. Most of the data is from animal studies in which IL-17 has been shown either to protect from tumor outgrowth or to promote tumor progression, depending on tumor origin and site.

Compared to IL-17, IL-22 has a more prominent effect on cell proliferation and differentiation, and, in animal models, several reports suggest that IL-22 favors tumor outgrowth of non-melanoma skin cancer and metastasis of colon and lung cancers (Perusina Lanfranca, 2016). In humans, increased expression of IL-22 or its receptor was reported to correlate with disease progression and decreased overall survival in cancers such as colorectal cancer (Tauriello, 2017).

Models of CAC are helping to clarify the mechanisms underlying accerrelated inflammation. Although the IL-22 and IL-17 pathways are involved in both the initiation and perpetuation of intestinal inflammation, it remains to be clarified whether these pathways can also be considered as triggers for the development of CAC.

Elevated levels of pro-inflammatory cytokines such as IL 17 are found in human CRCs, and numerous studies in mice show a key role for these cytokines in facilitating the survival and growth of CRC cells accordingly, cytokine blockers have been ascribed with robust antineoplastic effects in several CRC models (De Simone, 2013).

The tumor microenvironment is composed of a complex network of tumor cells, stromal cells and immune cells. Analysis of immune cells in the tumor tissues showed that further accumulation of Th17 and Th22 cells were relative to the paratumor tissues (Doulabi, 2018).

Finally, our findings suggest that increased intratumoral IL-17/IL-22 may play a significant role in colon cancer development and progression. Targeting of the pathway may have a suitable therapeutic efficacy in cancer therapy. A better comprehension of the immunological mechanisms of Th17, Th22 and CD4+ cells co-

© 2019 Discovery Publication. All Rights Reserved. www.discoveryjournals.org | OPEN ACCESS

producing IL-17/IL-22 in tumor immunity may help to develop novel and efficacious therapeutic strategies for colon cancer.

CONCLUSION

IL-17 and IL-22 are master components of a class of cytokines that is critically involved in epithelial homeostasis. The ablation of innate immune signalling in the gut as a therapeutic approach for intestinal pathologies should be clarified. During physiological conditions, the innate immune system is important for maintaining the homeostasis of the intestinal mucosa, but when altered it becomes the direct cause of the pathways underlying chronic inflammatory and neoplastic diseases. Our results suggest that the innate immune system participates to gut inflammation and colorectal cancer, suggesting that inflammatory responses can be common to both pathologies and contribute to the development of chronic inflammation associated cancer.

REFERENCES

1. Andoh A, Zhang Z, Inatomi O, Fujino S, Deguchi Y, Araki Y, et al. Interleukin-22, a member of the IL-10 subfamily, induces inflammatory responses in colonic subepithelial myofibroblasts. Gastroenterology. 2005;129(3):969-84.
2. Artis D, Spits H. The biology of innate lymphoid cells. Nature. 2015;517(7534):293-301.
3. Barrett JC, Hansoul S, Nicolae DL, Cho JH, Duerr RH, Rioux JD, et al. Genome-wide association defines more than 30 distinct susceptibility loci for Crohn's disease. Nat Genet. 2008;40(8):955-62.
4. Chan IH, Jain R, Tessmer MS, Gorman D, Mangadu R, Sathe M, et al. Interleukin-23 is sufficient to induce rapid de novo gut tumorigenesis, independent of carcinogens, through activation of innate lymphoid cells. Mucosal Immunol. 2014;7(4):842-56.
5. DeSimone, Role of TH17 cytokines in the control of colorectal cancer. Oncoimmunology.2013;11(2):99-110
6. Doulabi H, Analysis of Th22, Th17 and CD4+cells co-producing IL-17/IL-22 at different stages of human colon cancer. Biomed Pharmacother. 2018 Jul;103:1101-1106. doi: 10.1016/j.biopha.2018.04.147
7. Geremia A, Arancibia-Carcamo CV, Fleming MP, Rust N, Singh B, Mortensen NJ, et al. IL-23-responsive innate lymphoid cells are increased in inflammatory bowel disease. J Exp Med. 2011;208(6):1127-33.
8. Grivennikov S, Karin E, Terzic J, Mucida D, Yu GY, Vallabhapurapu S, 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):103-13.
9. Gwela A, Siddhanathi P, Chapman RW, Travis S, Powrie F, Arancibia-Carcamo CV, et al. Th1 and Innate Lymphoid Cells Accumulate in Primary Sclerosing Cholangitis-associated Inflammatory Bowel Disease. J Crohns Colitis. 2017;11(9):1124-34.
10. Hue S, Ahern P, Buonocore S, Kullberg MC, Cua DJ, McKenzie BS, et al. Interleukin-23 drives innate and T cell-mediated intestinal inflammation. J Exp Med. 2006;203(11):2473-83.
11. Jiang R, Tan Z, Deng L, Chen Y, Xia Y, Gao Y, et al. Interleukin-22 promotes human hepatocellular carcinoma by activation of STAT3. Hepatology. 2011;54(3):900-9.
12. Kamanaka M, Huber S, Zenewicz LA, Gagliani N, Rathinam C, O'Connor W, Jr., et al. Memory/effector (CD45RB(lo)) CD4 T cells are controlled directly by IL-10 and cause IL-22-dependent intestinal pathology. J Exp Med. 2011;208(5):1027-40.
13. Kirchberger S, Royston DJ, Boulard O, Thornton E, Franchini F, Szabady RL, et al. Innate lymphoid cells sustain colon cancer through production of interleukin-22 in a mouse model. J Exp Med. 2013;210(5):917-31.
14. Kortylewski M, Xin H, Kujawski M, Lee H, Liu Y, Harris T, et al. Regulation of the IL-23 and IL-12 balance by Stat3 signaling in the tumor microenvironment. Cancer Cell. 2009;15(2):114-23.
15. Liang SC, Tan XY, Luxenberg DP, Karim R, Dunussi-Joannopoulos K, Collins M, et al. Interleukin (IL)-22 and IL-17 are coexpressed by Th17 cells and cooperatively enhance expression of antimicrobial peptides. J Exp Med. 2006;203(10):2271-9.
16. Muranski P, Boni A, Antony PA, Cassard L, Irvine KR, Kaiser A, et al. Tumor-specific Th17-polarized cells eradicate large established melanoma. Blood. 2008;112(2):362-73.
17. Numasaki M, Fukushi J, Ono M, Narula SK, Zavodny PJ, Kudo T, et al. Interleukin-17 promotes angiogenesis and tumor growth. Blood. 2003;101(7):2620-7.
18. Perusina Lanfranca M, Lin Y, Fang J, Zou W, Frankel T. Biological and pathological activities of interleukin-22. J Mol Med (Berl). 2016;94(5):523-34.
19. Spits H, Artis D, Colonna M, Diefenbach A, Di Santo JP, Eberl G, et al. Innate lymphoid cells--a proposal for uniform nomenclature. Nat Rev Immunol. 2013;13(2):145-9.
20. Tanaka T, Kohno H, Suzuki R, Yamada Y, Sugie S, Mori H. A novel inflammation-related mouse colon carcinogenesis model induced by azoxymethane and dextran sodium sulfate. Cancer Sci. 2003;94(11):965-73.
21. Tauriello DV, Calon A, Lonardo E, Batlle E. Determinants of metastatic competency in colorectal cancer. Mol Oncol. 2017;11(1):97-119.
22. Thompson CL, Plummer SJ, Tucker TC, Casey G, Li L. Interleukin-22 genetic polymorphisms and risk of colon cancer. Cancer Causes Control. 2010;21(8):1165-70.
23. Tosolini M, Kirilovsky A, Mlecnik B, Fredriksen T, Mauger S, Bindea G, et al. Clinical impact of different classes of infiltrating T cytotoxic and helper cells (Th1, th2, treg, th17) in patients with colorectal cancer. Cancer Res. 2011;71(4):1263-71.
24. Witte E, Witte K, Warszawska K, Sabat R, Wolk K. Interleukin-22: a cytokine produced by T, NK and NKT cell subsets, with importance in the innate immune defense and tissue protection. Cytokine Growth Factor Rev. 2010;21(5):365-79.
25. Wu S, Rhee KJ, Albesiano E, Rabizadeh S, Wu X, Yen HR, et al. A human colonic commensal promotes colon tumorigenesis via activation of T helper type 17 T cell responses. Nat Med. 2009;15(9):1016-22.

Ethical approval

All procedures in this study were performed in accordance with the ethical standards of the institutional and/or national research committee and the 1964 Helsinki Declaration and its amendments or comparable ethical standards (institutional ethics committee code: 278.10.02.2018).

Financial Support

The financial support was from Turkish Gastroenterology Association.

Conflict of Interest

None of the authors have conflict of interest.

Article History

Received: 03 March 2019
Accepted: 11 April 2019
Published: May-June 2019

Citation

Elif Saritas Yuksel, Firdevs Topal, Özlem Gür, Fatih Topal, Cengiz Karahanlı, Berna Uran. IL-17 and IL-22 in colitis associated colorectal cancer in mice. *Medical Science*, 2019, 23(97), 375-380

Publication License

This work is licensed under a Creative Commons Attribution 4.0 International License.

General Note

Avoid print-versions. If required, article is recommended to print as color digital version in recycled paper. *Save trees, save nature*

© 2019 Discovery Publication. All Rights Reserved. www.discoveryjournals.org | OPEN ACCESS