http://www.medscidiscovery.com

OPEN ACCESS JOURNAL ISSN: 2148-6832

MSD Medical Science and Discovery
2020; 7(4):466-9

Research Article Doi: 10.36472/msd.v7i4.368

# TNF-alpha Induces Pro-Inflammatory Factors in Colorectal Cancer Microenvironment

**Ahu Pakdemirli[1]*, Gizem Calibasi Kocal[2]**

## Abstract

**Objective:** The tumor microenvironment has a crucial role in organizing cancer malignancy, progression, drug resistance and survival. It consists of cellular and non-cellular components. These non-cellular components such as cytokines, extracellular matrix, growth factors and metabolites are responsible for shifting the action from pro-cancer to anti-cancer effects. Twenty percent of all cancers occur in association with chronic inflammation via cytokines. Even cancers that are not caused by chronic inflammation, present high levels of cytokine expression pattern in their tumor microenvironment. Tumor necrosis factor-alpha (TNF-α) and some interleukins are characterized as pro-tumorigenic cytokines and they were involved in cancer by presenting their ability to activate the oncogenic transcription factors. The aim of this study is to evaluate the remodeling of colorectal cancer tumor microenvironment by TNF-α.

**Material and Methods:** TNF-α (5ng/ml) was applied to HT-29 colorectal cancer cells, then human soluble factors were determined by using Human Cytokine Group 1, 8 plex Panel (Bio-Rad Laboratories Inc. USA) and Magpix Luminex instrument and xPONENT software (version 4.2, Luminex Corp, Austin, Texas, US). The results were normalized to total protein concentration estimated via Bradford assay.

**Results:** Current research highlights the effect of TNF-α on the tumor microenvironment. Interleukin-6 and interleukin -8 soluble factors were higher in TNF-α treated colorectal cancer cells when compared with untreated control group.

**Conclusion:** The results of the study show that TNF-α is responsible for elevating the levels of interleukin-6 and interleukin-8, which are associated with inflammation in the tumor microenvironment.

**Key words:** Colorectal Cancer, Tumor Microenvironment, Cytokines, TNF-α, Interleukin-6, Interleukin-8

## Introduction

Colorectal cancer is the third most common cancer in males, while it is in the second most occurring cancer in females. In 2018, 1.8 million newly diagnosed colorectal cancer patient and approximately 861.000 deaths related to colorectal cancer are recorded by the World Health Organization. (GLOBOCAN, 2018) There are plenty of factors that cause colorectal carcinogenesis. One of these factors is a chronic inflammation that could trigger angiogenesis, evading from apoptosis, gene mutations, cell proliferation, epigenetic changes related to cancer development. Despite thorough proofs signifying a crucial role for inflammation colorectal cancer promotion and progression, still, there is comparably little knowledge on inflammation-associated microenvironmental alteration related to neoplasia/hyperplasia development and its progression through invasive colorectal adenocarcinoma (1).

The tumor microenvironment (TME) has a crucial role in organizing of cancer malignancy, progression, drug resistance and, survival, etc. Tumor genotype and phenotype are related to varying of cellular and non-cellular components in TME. The cellular components in TME are immune cells, adipocytes, cancer-associated fibroblasts, pericytes, etc. The non-cellular secreted elements of heterogeneous TME consist of cytokines, DNA, RNA, growth factors, metabolites, matricellular proteins, etc. These non-cellular substances are regulating numerous ways which providing cancer survival and progression via numerous growth signals, metabolites, energy, drug resistance-related environment, evading immune surveillance. These secreted components which are responsible for shifting the action from pro-cancer to anti-cancer effects are considered as novel targets in drug resistance and cancer therapeutics (2).

**Received** 25-03-2019 **Accepted** 28-04-2020 **Available Online** 29-04-2020 **Published** 30-04-2020
1 University of Health Sciences, Gulhane School of Medicine, Department of Physiology, Kecioren, Ankara, TR
2 Dokuz Eylul University, Institute of Oncology, Department of Translational Oncology, Inciralti, Izmir, TR
*** Corresponding Author:** Ahu Pakdemirli **E-mail:** ahu@pakdemirli.com

Almost 20% of all cancer appears in association with chronic inflammation and infection. Even if those cancers do not arise as a result of inflammation show a wide range of inflammatory infiltrates with increased cytokine expression levels in TME. (3) Cytokines elevate two-way interaction through paracrine signaling between cancer-associated cells in the environment and tumor cells. (4-6) A specific number of those cytokines with different functions such as interleukins (IL), tumor necrosis factor family, TGF-beta family of proteins and interferon family exist in the TME. Cytokines could take part in effect the tumor formation by acting indirectly by stimulating inflammatory cell type and directly as a growth-promoting factor on tumor cells. (7) Tumor necrosis factor-alpha (TNF-α) is one of those inflammatory mediators that is induced in carcinogenesis, via taking a part of chronic inflammatory diseases. (8) Other pro-inflammatory cytokines with a typical pro-tumorigenic effect are IL-6 and 8. Elevated serum IL-6 and 8 levels were discovered in patients with systemic cancers as compared to patients with benign diseases or healthy controls. (9-10)

In the current study, TNF- α induced pro-inflammatory factors interleukin 6 and 8 have been evaluated as inflammation modulators in the tumor microenvironment.

## Material and Methods

### Cell Culture

HT-29 cell line (CCL-247, ATCC, Rockville, CT, USA) was grown in McCoy's 5A media supplemented with 10% FBS, 2mM L-Glutamine at 37o C in a humidified incubator of %5 CO2. Cells were exposed to 5ng/ml TNF-α (Sigma, St Louis, Missouri, USA) for 48 hours, then soluble factors were measured.

### Analysis of Soluble Factors via Multiplexing Assay

According to Bio-Plex Pro assays instruction manual, Human Cytokine Group 1, 8 plex Panel (Bio-Rad Laboratories Inc. USA) was achieved. At first 50 µl of 1x beads were added to the assay plate then wells were washed 2 times with 100 µl wash buffer (Bio-Rad Laboratories Inc. USA) which was provided with kit. After that 50µl of sample were added to each well.

Then the plate was incubated for an hour in dark at RT with shaking at 300 RPM. After incubation, The plates have been (Bio-Rad Laboratories Inc. USA) washed with wash buffer for three times. Subsequently, 25 µl antibody solution (Bio-Rad Laboratories Inc. USA) was added to each well for incubation for 30 min. After that the plate was washed with wash buffer for three times.

The 50 µl Streptavidin-PE (Bio-Rad Laboratories Inc. USA) was added to each well for incubation for 30 min. Finally, the plate was washed with 120 µl of assay buffer (Bio-Rad Laboratories Inc. USA) which was provided with kit.

The fluorescent signal was measured by a CCD imager and the concentrations of the analyte were determined with both Bio-Plex Manager and MAGPIX®- Luminex xPONENT software (Bio-Rad Laboratories Inc. USA).

**Statistical analysis:** SPSS V.15.0 (SPSS, Chicago, Illinois, USA) was used for the data analysis. The mean ± the SE (standard error) was used for numerical values found in the figures and text.

A non-parametric Mann Whitney U test was used to determine the statistical significance. All p-values <0.05 were considered statistically significant.

## Results

In this study remodeling of colorectal cancer tumor microenvironment after TNF-α induction was evaluated. The graph showed that significantly elevated IL-6 and 8 levels after 48 hours of TNF-α exposure (Figure 1) (p-value <0.05). Data for each group with mean and standard error showed on Table 1.

In TNF-α treated group, IL-6 amount was measured as 27 pg/ml, while untreated group IL-6 amount was 10 pg/ml. Overall IL-6 level was increased 2.7 fold in TNF-α treated group. IL-8 level was elevated more than IL-6. In TNF-α treated group, IL-8 amount was measured as 595 pg/ml, while untreated group was 23 pg/ml. IL-8 level was increased 25.9 fold when compared with untreated control group.

**Table 1.** Stimulated levels of IL-6 and IL-8 cytokines (pg/ml) after a 48-h exposure with TNF-α.

| | Interleukin-6 | | Interleukin-8 | |
|---|---|---|---|---|
| | Untreated | TNF-α treated | Untreated | TNF-α treated |
| **Mean (pg/ml)** | 10 | 27 | 23 | 595 |
| **Min-Max** | 8 - 12 | 24 - 30 | 18 - 28 | 493 - 697 |
| **SEM** | 0.9522 | 1.299 | 2.380 | 54.73 |
| **P-Value** | 0.0003 | | < 0.0001 | |
| **Fold change** | 2.7 | | 25.9 | |

http://dx.doi.org/10.36472/msd.v7i4.368

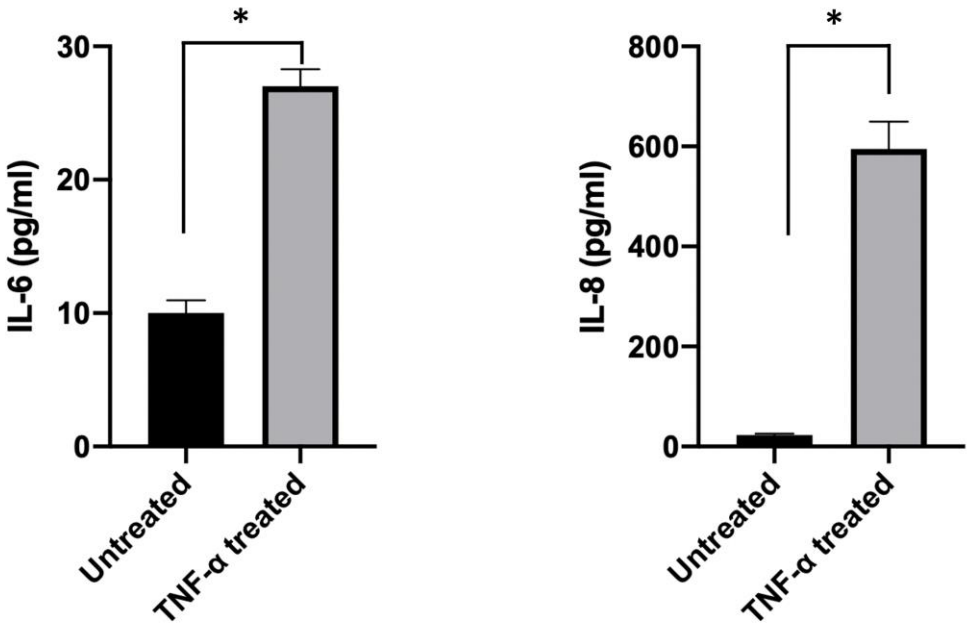


**Figure 1.** The expression pattern of IL-6 and IL-8 soluble factors in TNF-α treated and control (untreated) group.

## Discussion

Inflammation in cancer is guided by chemokines, cytokines and soluble factors. These factors are secreted by tumor cells or tumor microenvironment secretes them by their recruited cells. Cytokines encourage tumor cell growth, differentiation, and survival. Most of the cytokines play roles in metastasis, epithelial-mesenchymal transition, angiogenesis, invasion, proliferation via transforming the intestinal epithelial cells (IECs) and, apoptosis. Cytokines trigger cancer-associated fibroblasts to secrete growth factors that inflect tumor microenvironment (1). Inflammatory (or pro-inflammatory) cytokines are secreted by macrophages, helper T cells and other certain cell types that develop inflammation. Pro-inflammatory interleukins consist of IL-1, IL-2, IL-6, IL-8, and TNFα (11). Fundamental secretion of the pro-inflammatory interleukins leads to an inflammation in the intestine and is thought to increase the risk for colon and rectal cancer development (12). Increased expression of IL-6 is associated with an expanded risk of colorectal adenomas. IL-6 is also a strong stimulator of colon cancer cell growth and proliferation. This growth implicates the expansion of other cancer cell lines and primary tumors.

TNF-α and IL-6 or transcription factors of these cytokines, such as NF-κB and STATs, certainly appear as potential targets for anticancer therapy (13). The tumor-promoting features of TNF-α are presumably linked to its capacity to activate NF-κB and AP-1 signaling pathways that provoke cell proliferation and survival (14). In our study, it was observed that TNFα increases the secretion levels of IL-8 and IL-6 on HT-29 colorectal cancer cell line. Similarly, Edwardson et al. evaluated that an increased amount of cytokines expands the effectiveness of the drug in the cell microenvironment (15).

## Conclusion

TNF- α and IL-6 are presumably the best identified pro-tumorigenic cytokines and they were primarily suspected to be involved in cancer development because of their ability to activate the oncogenic transcription factors STAT3 (IL-6), AP-1 (TNF) and NF-κB in epithelial cells.

Even though preclinical data are highly supportive about the effect of drugs that target pro-inflammatory tumor microenvironment on tumor cell growth and survival, clinical trials will need to arrange to approve their impact in patients. Also, these drugs should be determined whether they will be beneficial as single agents or should they be used in combination with standard cytotoxic therapies (13).

**Acknowledgments:** We sincerely thank to Ezgi DAŞKIN, Tolga SEVER and Aslıhan KARADAG for their support during the entire study process

**Conflict of interest and financial disclosure:** The authors declare that there is no conflict of interest and financial relationships.

**Author's contiributions:** AP, GCK: Design of study, Invitro studies, Data analyzes and statistics, Revisions

**Ethical issues:** Author declare, originality and ethical approval of research. The study was conducted under defined rules by the Local Ethics Commission guidelines and audits.

## References

1. Patel H, Nilendu P, Jahagirdar D, Pal JK, et al. Modulating secreted components of tumor microenvironment: A masterstroke in tumor therapeutics. Cancer Biol Ther. 2018;19(1): 3–12.

2. Colotta F, Allavena P, Sica A, Garlanda C, et al. Cancer-related inflammation, the seventh hallmark of cancer: links to genetic instability. Carcinogenesis. 2009;30:1073-81.

3. Mantovani A, Allavena P, Sica A, et al. Cancer-related inflammation. Nature. 2008;454:436–44.

4. Matsumoto S, Hara T, Mitsuyama K, et al. Essential Roles of IL-6 Trans-Signaling in Colonic Epithelial Cells, Induced by the IL-6/soluble-IL-6 Receptor Derived From Lamina Propria Macrophages, on the Development of Colitis-Associated Premalignant Cancer in a Murine Model J Immunol. 2010;184: 1543–51.

5. Ernst M, Najdovska M, Grail D, et al. STAT3 and STAT1 mediate IL-11-dependent and inflammation-associated gastric tumorigenesis in gp130 receptor mutant mice. J Clin Invest. 2008;118: 1727–38.

6. Becker C, Fantini MC, Wirtz S, et al. IL-6 signaling promotes tumor growth in colorectal cancer. Cell Cycle. 2005;4:217–20.

7. Rigby RJ, Simmons JG, Greenhalgh CJ, et al. Suppressor of cytokine signaling 3 (SOCS3) limits damage-induced crypt hyper-proliferation and inflammation associated tumorigenesis in the colon. Oncogene. 2007;26:4833–41.

8. Popa C, Netea MG, Van Riel P. LCM, et al. The Role of TNF-alpha in Chronic Inflammatory Conditions, Intermediary Metabolism, and Cardiovascular Risk. J Lipid Res. 2007;48(4):751–62.

9. Kumari N, Dwarakanath, BS, Das A, Bhat AN. Role of interleukin-6 in Cancer Progression and Therapeutic Resistance. Tumor Biol. 2016;37(9):11553–72.

10. Bărbălan A, Streața I, Ivan ET, et al. Interleukin-8 mRNA Expression in Locally Advanced Colorectal Cancer Patients. Curr Health Sci J. 2017;43(3):209–13.

11. Bondurant KL, Lundgreen A, Herrick JS, et al. Interleukin Genes and Associations with Colon and Rectal Cancer Risk and Overall Survival. Int J Cancer. 2013;132(4):905-15.

12. Janos Terzić 1, Sergei Grivennikov, Eliad Karin, Michael Karin. Inflammation and Colon Cancer. Gastroenterology. 2010;138(6):2101-14.

13. Klampfer L. Cytokines, Inflammation and Colon Cancer. Curr Cancer Drug Targets. 2011;11(4):451-64.

14. Grivennikov SI, Karin M. Inflammatory Cytokines in Cancer: Tumour Necrosis Factor and Interleukin 6 Take the Stage. Ann Rheum Dis. 2011;70(1):104-08.

15. Edwardson DW, arissenti AM, Kovala AT. Chemotherapy and Inflammatory Cytokine Signalling in Cancer Cells and the Tumour Microenvironment. Adv Exp Med Biol. 2019;1152:173-215.