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# The role of NF-κB p65 and hsp70 in colorectal adenocarcinomA

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***Abstract*---**Free radicals and inflammation in tumor microenvironment are important aspects of tumor biological activity. They activated NF-κB p65 transcription factor pathway. HSP70 is chaperone protein that regulated host of imune response to cancer cells. NF-κB p65 and HSP70 expression is associated with poor prognosis and new targeted therapy in colorectal adenocarcinoma. This study aims to analyze correlation between NF-κB p65 expression in macrophages and HSP70 in tumor cells with various T stages of colorectal adenocarcinoma. This cross-sectional study was performed on 48 paraffin blocks from colorectal adenocarcinoma at the Anatomical Pathology Laboratory of Dr. Soetomo General Academic Hospital Surabaya from January 2015 to December 2019. The samples were divided into four groups based on T stages of colorectal adenocarcinoma. Immunohistochemical staining was performed to detect NF-κB p65 expression in nucleus or cytoplasm of macrophages and HSP70 in cytoplasm of tumor cells. There was no significant difference of NF-κB p65 expression percentage in macrophages at four groups (p=0.31). There was significant differences of HSP70 expression

percentage in tumor cells at four groups (p=0.014). There was no correlation between NF-κB p65 expression in macrophages and HSP70 expression in tumor cells with various T stages of colorectal adenocarcinoma (rs=0.05, p=0.97).

***Keywords***---T Stages, colorectal adenocarcinoma, NF-κB p65, HSP70.

## Introduction

Colorectal adenocarcinoma is a malignancy which serves as the third most common cancer in the world. The estimated incident of carcinoma colorectal reached at 2.2 million cases and numbers estimated at 1.1 million deaths by 2030 (Marjaneh et al., 2019). The data were taken from the information center of The Indonesian Ministry of Health in 2018, thus, they stated that the incident of carcinoma colorectal is a malignancy which serves as the third most common cancer in Indonesia (Kemenkes RI, 2017). The age-standardized incidence rates of colorectal adenocarcinoma per 100.000 populations in Indonesia were 15.9 for males (the second most cancer occurred after lung cancer) and 10.1 for females (the third most cancer occurred after breast and cervix uterine cancer) (Mastutik et al., 2016).

Furthermore, the standard for the therapy in colorectal adenocarcinoma is surgery. Performing surgery which is combined with regimen chemotherapy especially at an advanced stage is an important component therapy. It is an effective way of the assessment relating to the clinical patients with colorectal adenocarcinoma. Recurrence and metastases are the main causes of the death despite of the combination therapy. Therefore, there is an urgent medical needed for research that comprehends the demand of the new alternative therapeutic approaches to treat colorectal adenocarcinoma properly (Fenoglio et al., 2008; Marjaneh et al., 2019).

Microenvironment also serves as the important role to accelerate the colorectal adenocarcinoma. Tumor cells can cause imbalance among free radicals and are known as the oxidative stress. Tumor cells trigger formation of free radicals, especially ROS that ultimately cause oxidative DNA damage to emerge hypoxia cells (Sudiana, 2011). Tumor cells, which have hypoxia, will activate HSP70 for hinder damage membrane cell. Thus, the cell tumor enhances permanently and activate the track of hypoxia-inducible factor- 1α (HIF -1α) that beneficial to the activate macrophages through arginase pathway in the microenvironment tumors (Sreevalsan and Safe, 2013; Rath et al., 2014; Albakova et al., 2020). Macrophages is an important immune response mediator of tumour growth. Macrophages consist of pro-inflammatory and anti-inflammatory which will induce expenditure cytokines that activate the track of NF-kB. Therefore, they produce TNF-α which can bind to the receptor i.e. TNFR-1. It generates the hinder proliferation of tumor cells and the extent of TNFR-

2 triggers proliferation of tumor cells that influence various T stages tumor cells (Henze and Mazzone, 2016; Idriss and Naismith, 2000).

Nuclear factor-kappa B (NF-κB) is the transcription factors that mediate cytoplasmic or nuclear signaling pathways. They serve as the important role in the regulation of the cell proliferation and survival life cells (Park and Hong, 2016). The activation of NF-kB P65 has a correlation with control of apoptotic pathways, cell proliferation, angiogenesis, invasion and metastasis cell tumors. In the phase II study, it was found that there was a correlation between NF-B activation and resistance to treatment in advanced to colorectal cancer cases. This is because the enhancement of NF-kB activation could cause resistance to chemotherapy or radiotherapy. Furthermore, the inhibition of the NF-Kb p65 pathway can sensitize colon cancer cells to chemotherapy or radiotherapy and provide a more effective strategy for new targeted therapy cancer colorectal (Plewka et al., 2018; Soleimani et al., 2019).

Heat shock protein 70 (HSP70) is a group of chaperone molecules that mediate intracellular processes including protein folding, modification and translocation, as well as regulation of apoptosis and survival life cells. On the other hand, HSP70 extracellular function stimulates the response immunity to become innate and adaptive. Tumor cells express higher HSP70 compared to normal cells. HSP70 is responsible for the process of tumorigenesis and as a resistance to drug chemotherapy. The enhancement of HSP70 expression has been found in cancer colorectal and related with a poor prognosis (Marjaneh et al., 2019). HSP inhibitors are being evaluated in clinical trials on several patients with cancer colorectal that express an excessive HSP70 on the surface cell cancer colorectal. The former facilitates tumor migration and invasion (Kumar et al., 2016; Li Zongwei et al., 2013). In contrast, the inhibition of HSP70 reduces tumor size and increases response to therapeutic strategies of cancer, thus, HSP70 inhibitors can be one of the choices therapy for cancer colorectal (Marjaneh et al., 2019).

NF-kB pathway on macrophages and HSP70 on tumor cells can influence invasion and migration of the tumor cells which correlate to the various stages of T in colorectal adenocarcinoma. According to The American Joint Committee on Cancer (AJCC) or International Union Against Cancer (UICC), the tumor-node- metastasis (TNM) staging system relates to predict survival, patient prognosis and the implementation of therapeutic regimens for colorectal cancer patients. One component was taken from the staging system i.e. T stage was based on the depth of invasion of the primary tumor. This T stage has been recognized as an important factor for predicting outcomes of patients (Athanasakis et al., 2018).

**Method Study Design**

This study was analytic observational research with a cross-sectional approach performed on the 48 formalin fixed paraffin embedded (FFPE) tissues from colorectal adenocarcinoma patients at the Anatomical

Pathology Laboratory of Dr. Soetomo General Academic Hospital Surabaya from January 2015 to December 2019. The samples were taken using purposive sampling technique and divided into four groups based on T stages of colorectal adenocarcinoma which consist of T1=2, T2=14, T3=21, T4=11 formalin fixed paraffin embedded (FFPE). The data were recorded by reviewing patients' medical reports. In addition, this study has been permitted by the Committee of Health Research Ethic at Dr. Soetomo General Academic Hospital Surabaya (0715/LOE/301.4.2/XII/2021).

**Immunohistochemistry Staining**

Immunohistochemistry staining was performed to detect the expression of NF- κB p65 and HSP70. Block samples of FFPE tissue were cut into four □m sections with Leica microtome into slides, deparaffinized three times with xylol for five minutes each, and rehydrated through graded alcohol. Moreover, the slides were warmed using Target Retrieval Solution (TRS) or buffer citrate by decloaking chamber with pH 6.0 and temperature 95 °C for 20 minutes. Then, background snipper was dripping for fifteen minutes. The tissue sections were incubated with primary antibody which consist of monoclonal antibodies for NF-kB p65 (dilution 1:100; GeneTex) and IL-10 (dilution 1:600; Santa Cruz Biotechnology) for sixty minutes, followed by the first secondary antibody (trekkie link) for 20 minutes and second secondary antibody (HRP label) for 10 minutes at room temperature. The sections were counterstained with diaminobenzidine and hematoxylin for 10 minutes in a room with comprehensive temperature and were dehydrated with alcohol.

**Evaluation of Immunohistochemical Expression**

Positive results were obtained if NF-kB p65 staining expressed in cytoplasm or nuclear macrophages and HSP70 staining expressed in cytoplasm tumor cells. Two pathologists evaluated all samples in a blinded fashion. Any discordance was solved by the inter-observer agreement. If there was a score gap >20 then it would be read by a third pathologist. The evaluation expression of NF- kB p65 was performed with method sum the nucleus or cytoplasm stained positive in macrophages which shared amount in whole macrophages and counted in percentage (%) and rating HSP70 expression with method sum up cytoplasm stained positive in tumor cells which shared amount in whole tumor cells and counted in percentage (%).

**Statistical analysis**

SPSS software was used for statistical analysis in this research and further analysis was tested using Annova and Kruskal-Wallis test. The difference of NF-kB p65 and HSP70 expression in any T stages of colorectal adenocarcinoma was considered significant if $p<0.05$. The correlation between NF-kB p65 and HSP70 expression was assessed using the Spearman correlation test. Moreover, significant correlation was indicated by p value $<0.05$.

**Results and Discussions**

**NF-kB p65 Expression in Macrophages with Various T Stages of Colorectal adenocarcinoma**

The data obtained were normally distributed ($p > 0.05$) in this study. Thus, the difference of NF-kB p65 expression in various T stages of adenocarcinoma was analyzed by the Anova test. Analysis result statistics show there was no significant difference among expression of NF-B p65 in macrophages in various T stages of colorectal adenocarcinoma (p value=0.31) (Table 1 and Figure 1).

Table 1
NF-kB p65 expression in macrophages with various T stages of colorectal adenocarcinoma

| T stages | n (%) | Average | p value |
|---|---|---|---|
| T1 | 2 (4%) | 57,5 | |
| T2 | 14 (29%) | 58,2 | |
| | | 0,31 | |
| T3 | 21 (44%) | 52,5 | |
| T4 | 11 (23%) | 48,5 | |

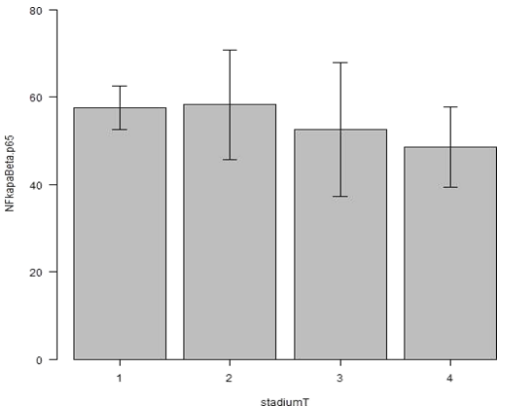


Image 1. There is no significant difference in NF-kB p65 expression in macrophages with various T stages of colorectal adenocarcinoma.

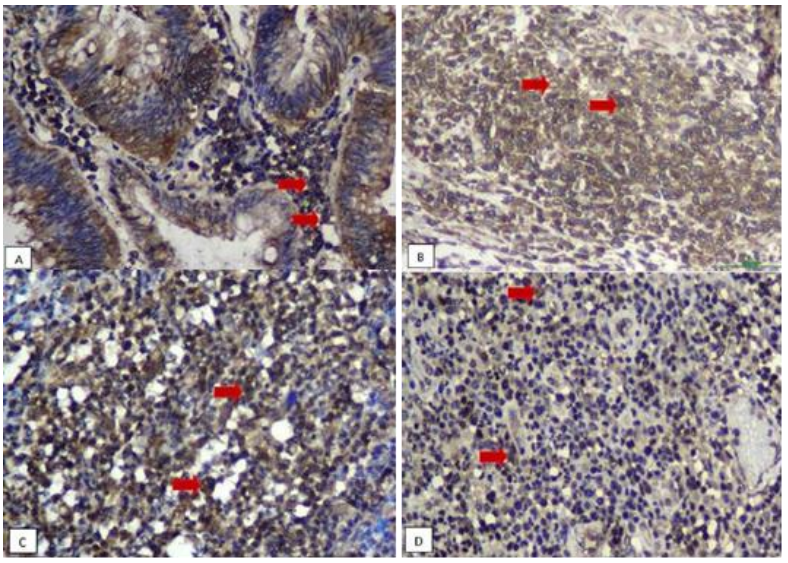


Figure 2. Immunohistochemical expression of NF-kB p65 in macrophages with various T stage colorectal adenocarcinoma. A: Expressed in 61% cytoplasm or nucleus of macrophages in T1 stage (arrow) (200x); B: Expressed in 79% cytoplasm or nucleus of macrophages in T2 stage (arrow) (400x); C: Expressed in 75% cytoplasm or nucleus of macrophages in T3 stage (arrow) (400x); and D: Expressed in 42% cytoplasm or nucleus of macrophages in T4 stage (arrow) (400x)

The correlation between NF-kB p65 expression in macrophages with various T stages of colorectal adenocarcinoma was analyzed by Spearman's non-parametric correlation test. The results showed that there was no significant correlation between NF-kB p65 expression on macrophages and various T stages of colorectal adenocarcinoma with p value = 0.052 and value coefficient correlation negative at 0.282. The results of the Spearman correlation test were shown in table 2.

Table 2
Spearman correlation test results between NF-kB p65 expression in macrophages with various T stages of colorectal adenocarcinoma

| | | NF-kB p65 expression |
|---|---|---|
| T stage colorectal adenocarcinoma | $r_s$ | -0,282 |
| | p | 0,052 |
| | n | 48 |

**HSP70 Expression in Tumor Cells with Various T Stages of Colorectal adenocarcinoma**

The data obtained were not normally distributed ($p < 0.05$) so the difference of HSP70 expression in various T stages of adenocarcinoma was analyzed by Kruskal-Wallis test. Analysis result statistics showed that

there was significant difference among HSP70 expression in tumor cells in various T stages of colorectal adenocarcinoma with p value = 0.014. HSP70 expression data in tumor cells in various T stages of colorectal adenocarcinoma presented in table 3 and figure 3

Table 3
HSP70 expression in tumor cells with various T stages of colorectal adenocarcinoma

| T stages | n (%) | Median | p value |
|---|---|---|---|
| T1 (a) | 2 (4%) | 45,0 | |
| T2 (b) | 14 (29%) | 70,5 | |
| T3 (c) | 21 (44%) | 83,0 | |
| T4 (c) | 11 (23%) | 82,0 | 0,014 |

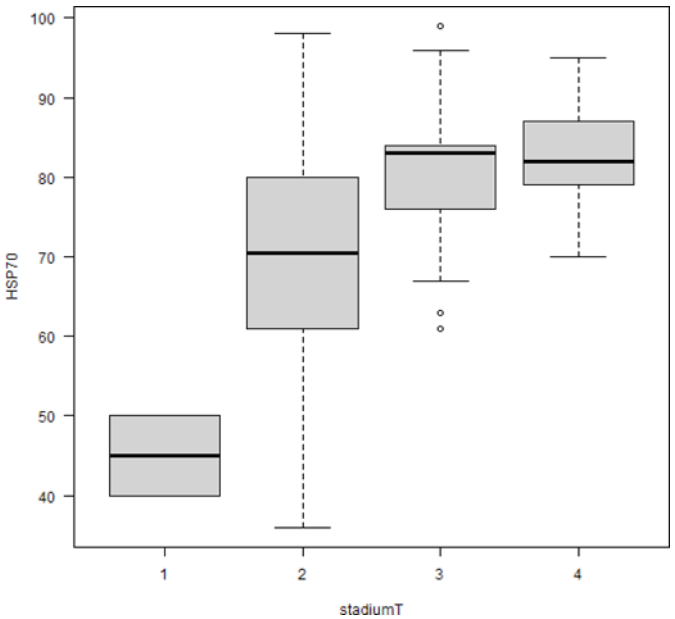


Figure 3. There is significant difference in HSP70 expression in tumor cells with various T stages of colorectal adenocarcinoma

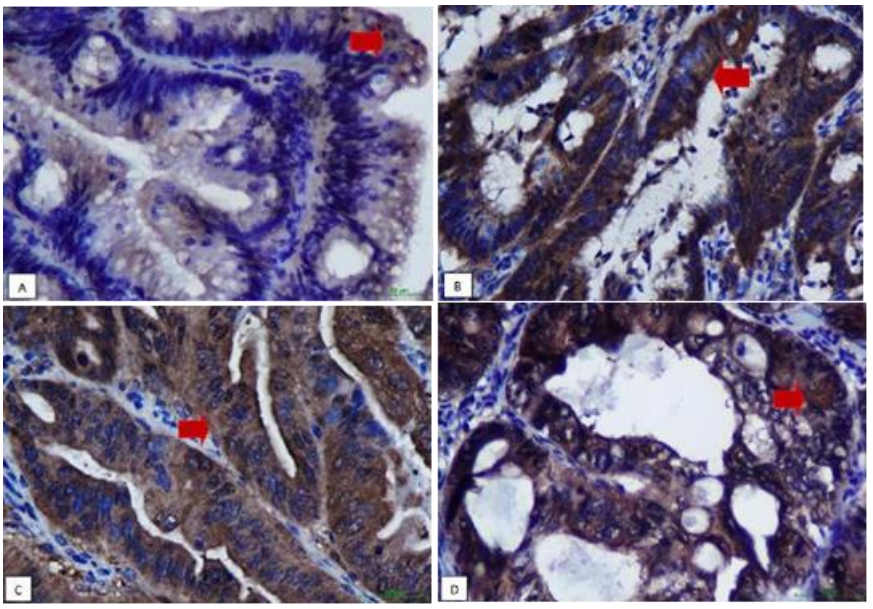

Figure 4. Immunohistochemical expression of HSP70 in tumour cells with various T stage of colorectal adenocarcinoma. A: Expressed in 40% cytoplasm of tumor cells in T1 stage (arrow) (400x); B: Expressed in 74% cytoplasm of tumor cells in T2 stage (arrow) (400x); C: Expressed in 99% cytoplasm of tumor cells in T3 stage (arrow) (400x); and D: Expressed in 89% cytoplasm of tumor cells in T4 stage (arrow) (400x).

The correlation between HSP70 expression in tumor cells with various T stages of colorectal adenocarcinoma was analyzed by Spearman's non-parametric correlation test. The test results showed that there was significant correlation between HSP70 expression in tumor cells and various T stages of colorectal adenocarcinoma with p value = 0.003 and value coefficient correlation positive at 0.426. Spearman correlation test results were shown in table 4.

Table 4
Spearman correlation test results between HSP70 expression in tumor cells with various T stages of colorectal adenocarcinoma

| | | HSP70 expression |
|---|---|---|
| T stages colorectal adenocarcinoma | $r_s$ | 0,426 |
| | p | 0,003 |
| | n | 48 |

**Correlation Expression NF -kB p65 in Macrophages and HSP70 in tumor cells with Various T Stages of Colorectal adenocarcinoma**

The Spearman correlation test was conducted to acknowledge the correlation between NF-kB p65 expression in macrophages and HSP70 in tumor cells in various T stages of colorectal adenocarcinoma. Analysis

results showed that there was no significant correlation between NF-k-B p65 in macrophages and HSP70 in tumor cells with p value = 0.97 and value coefficient correlation positive at 0.005. By this mean NF-kB expression in macrophages does not necessarily affect HSP70 expression in tumour cells at various T stages of colorectal adenocarcinoma. The results of the Spearman correlation test were shown in table 5.

Table 5
There is no significant correlation among NF-kB p65 expression in macrophages with HSP70 in tumor cells

| | | NF-kB p65 expression |
|---|---|---|
| HSP70 expression | $r_s$ | 0,005 |
| | p | 0,97 |
| | n | 48 |

**Discussions**

**NF-kB p65 Expression in Various T Stages of Colorectal adenocarcinoma**

In this study, NF-kB p65 expression in all samples was presented in the macrophages cytoplasm or nuclei. This was in accordance with the study by Chorolombous et al who stated that the majority of patients of colorectal cancer expressed NF-kB-p65 (score 3) in macrophages (Chorolombous et al., 2009). NF-kB p65 expression in cytoplasm represents an inactive protein which binds to IkB, as well as active NF-kB p65, which has become a phosphorylated and released from IkB; however, still has not translocate to the nucleus yet. On the other hand, NF-kB-p65 expression in the nucleus represents the active protein. In determining whether there is NF-kB p65 protein in the cytoplasm in active form or not, Chorolombous et al investigated NLS expression of NF-kB-p65 at six patients expressing significant cytoplasmic NF-kB p65. Anti-NF-kB-p65 NLS antibodies recognize, specifically, overlapping epitopes with the NLS of the p65 subunit of the NF-kB heterodimer. The epitope is covered by I-kB binding and the anti-NF-kB-p65 NLS antibody will selectively bind with free I-kB and will be activated to form NF-kB-p65, therefore, excessive amount of NF-kB-p65 was found in active form in the cytoplasm (Chorolombous et al., 2009).

The statistics analyses were performed with Anova test showed that there were no differences between NF-kB p65 expression in various T stages of colorectal adenocarcinoma ($p = 0.31$). NF-kB p65 expression in macrophages tended to be high in all T stages of colorectal adenocarcinoma with the highest value found at T2 stage (58,2 %) and the lowest at T4 stage (48.5 %) respectively. Cell cancer will stimulate the microenvironment with activated macrophages. The result of NF-kB p65 expression in macrophages had no significant difference, and the expression tended to be high in all stages due to NF-kB was expressed by many types of macrophages. The M1 macrophages promote various type pro-inflammatory cytokines actively e.g. IL1β, IL6, TNF-α, and ROS. In

some studies, if proinflammatory macrophages activation was triggered by tumor cells that flow continuously, the pro-inflammatory cytokine can promote a malignant transformation. It supports the hypothesis that the extent of inflammation will promote malignant transformation that was coined by Rudolf Virchow in the 19th century, therefore, at higher stages, such as T3 and T4, NF-kB p65 expression in macrophages is considerably high (Zhong et al., 2018; Henze and Mazzone, 2016).

The evaluation of macrophages in this study was conducted with hematoxylin eosin staining so it was difficult to differentiate M1 macrophages and M2 macrophages, whereas, the purpose of this study is to evaluate the NF-kB p65 expressed by M1 macrophages. Jalbonski et al stated that CD38/Egr2-based flow cytometry examination can differentiate M1 and M2 macrophages better, compared with phenotype marker such as iNOS, arginase-1 and classic CD206. CD38, Gpr18 and Fpr2 are the newest M1 markers, whereas Egr2 and C-MYC are the marker of M2 (Jalbonski et al, 2015). The dynamic changes occurred in the microenvironment during transition phase from early to advanced stages of cancers have a fluctuative results of M1 and M2 macrophages, therefore M1 and M2 macrophages were also presented in the advanced stage of cancer. M1 macrophages activation is related to the formation of necrotic area, whereas M2 activation is related with angiogenesis and cells proliferation in high quantities. Moreover, the balance among phenotype on different macrophages is a clear impact to tumor progression and mechanisms that could be a target of the important therapy (Mancino and Lawrence, 2010). There were no significant differences resulted in NF-kB p65 expression in macrophages in all stages of colorectal adenocarcinoma because of the influence of the TNF-α expression. The canonical pathway begins with extracellular stimuli such as oncogenic molecules that will promote the tumor necrosis factor receptor (TNFR) with its specific ligand is TNF-α. Macrophages produced TNF-α and activated the inflammatory process during tumor promotion. NF-kB is a downstream of TNF-α and inflammation cytokines; however, other cytokines were the main mediators in the tumor microenvironment which triggers the tumor activity.

TNF-α was binding with TNFR to activate NF-kB signaling pathway which correlated with invasion and migration of tumor cell, and forming the IKKα/β/ɣ complex. IKKβ phosphorylates IκB which was the inhibitor of NF-kB in the cytoplasm. NF-κB was inactivated by enveloping the nuclear localized sequence (NLS), blocking DNA binding and nuclear uptake of NF-κB (Soleimani et al., 2019), then induce degradation and trigger NF-kB release. As a result, NF-kB p65 translocated to the nucleus and induced gene expression which related with cells proliferation, cells survival and invasion. TNF-α as endogenous tumor-promoting factors can induce epithelial-mesenchymal transition (EMT) pathway, immunity response, and angiogenesis (Shi et al, 2018).

TNF can induce dead cells either necrotic or apoptotic cells. Furthermore, necrosis is marked with cells swelling, organelles damage, and cells lysis.

Apoptotic is considered as a process of the programmed death and driven by cells in biochemistry with different morphology. Apoptosis is marked with cells shringkage, formation of apoptotic bodies, and formation of DNA fragmentation among nucleosomes. Apoptosis is seen as a phenomenon where TNF-α mediated cytotoxicity occurs in the pathway of TNFR-1 signaling. TNFR-2 indirectly mediates cytotoxicity through endogenous TNF production and activation of autotrophic or paratrophic TNFR-1. TNF can also maintain cells survival through activation of NF-kB (Idriss and Naismith, 2000).

In this study, the correlation between NF-kB p65 expression and various T stages of colorectal adenocarcinoma was analyzed by Spearman Test showed no significant correlation between NF-kB p65 expression in macrophages and all of T stages of colorectal adenocarcinoma ($p = 0.052$). This was different with the study of Gonzalez-Quezada et al which stated that NF-kB has positive correlation not only on the depth tumor invasion but also correlated with lymph nodes and metastatic status (Gonzalez-Quezada et al., 2018). Shi et al reported that the translocated NF-kB heterodimer to nucleus will induce the gene expression related to cells proliferation, cells survival, invasion, EMT pathway, response immunity, and angiogenesis. NF-kB activation could induce EMT pathway, therefore NF-kB is a potential target for prevent invasion and migration of cell tumors induced by several agents. NF-kB is very important for TNF-α to induce EMT pathway. This study has portrayed that TNF-α induced invasion and migration were inhibited by drugs that inhibit NF-kB activation. For instance, vitamin D3 increases regulation of protein 1, which inhibits hepatocarcinogenesis by blocking the TNF-α induced activation of NF-kB. Crebanine can also inhibit cells invasion of lung adenocarcinoma by blocking the NF-kB regulated gene product (Shi et al., 2018).

**HSP70 Expression in various T stages of colorectal adenocarcinoma**

The statistics analyses were performed with Kruskal-Wallis test showed that there were significant differences between HSP70 expression in various T stages of colorectal adenocarcinoma ($p = 0.014$). HSP70 expression in tumor cells with the highest median value found at T3 stage (83 %) and T4 stages (82 %) while the lowest was at T1 stage (45 %) that caused by HSP70 affecting tumor cells survival pathway (Goa et al., 2021). In some humans cancer, HSP70 has been proven as the best prognostic factor and it was independent prognostic factor. The enhancement of HSP70 expression has been proven in colorectal cancer and related with a poor prognosis (Sherman and Gabai, 2015). In this study, HSP70 expression was lowest at T1 stage compared to T2, T3 and T4 stage, therefore, T1 stage of colorectal adenocarcinoma have the best prognosis. Overexpression of HSP70 significantly related with the overall survival and desease-free survival in patients with colorectal adenocarcinoma (Goa et al., 2021).

In this study, the correlation between HSP70 expression and various T stages of colorectal adenocarcinoma was analyzed by Spearman Test showed significant correlation between HSP70 in tumor cells and all of T stages of colorectal adenocarcinoma (p value = 0.003). This study results was accordance with Wang et al which showed that HSP70 expression at high level in tumor cells though without any stimulation and there is a possible correlation among HSP70 expression, growth and development tumor cells (Wang et al., 2005). This study showed that HSP70 and grp94 expression in colon cancer was higher than in surrounding normal tissue. HSP70 and grp94 expression in colon cancer with poor differentiate with metastases was higher than colon cancer with well differentiate without metastases. It showed that the enhancement of HSP70 and grp94 expression was likely to have connection with development, invasion, and metastasis of colon cancer (Wang et al., 2005).

HSP70 is a new therapeutic target, where the most active compound is HSP90/HSP70 inhibitor that induces apoptosis in cancer cells. HSP70 inhibition will reduce tumor size and increase response to therapeutic strategies for cancer. HSP70 inhibitor chaperone molecule will be one of the choices therapy in colorectal cancer (Marjaneh et al., 2019). Various HSP inhibitors are being evaluated in clinical trials, which there was an increase HSP70 expression in cancer cells found in patients with treatment. But, the accumulation of HSP70 can also reduce cell death, thereby decreasing the antitumor efficacy of HSP70 inhibitors. Treatment with chemotherapy drugs increases HSP70 expression and induces TGF-β signaling. Therapy combination of adenosine-derived inhibitors of HSP70, VER-155008, and 17-AAG induces apoptosis in HCT116 carcinoma colon, so that the anti-cancer activity of 17-AAG will increase in cancer colon cells with combination therapy HSP70 inhibitor (Kumar et al., 2016).

**Correlation Expression of NF -B p65 in Macrophages and HSP70 in Tumor Cells with Various T stages of Colorectal adenocarcinoma**

The correlation between NF-kB p65 expression in macrophages and HSP70 in tumor cells with various T stages of colorectal adenocarcinoma with Spearman test showed that there was no significant correlation between NF-kB p65 in macrophages and HSP70 in tumor cells (p value = 0.97) and coefficient correlation value is positive 0.005. This means that NF-κB expression will not affect HSP70 expression in all of T stages of colorectal adenocarcinoma. In this study, there was no significant correlation between NF-kB p65 expression in macrophages and HSP70 expression in tumor cells in the pathway inflammation. It could be caused by the presence survival pathway. Moreover, Kumar et al stated that HSP70 in the cytosol can inhibit NF-kB p65 expression and membrane-bound HSP70 can induce this transcription factor. Response of similar stimuli in cytosol and membrane-bound HSP70 were stimulated. HSP70 expression in endothelium cells which stress-induced apoptosis facilitated TNF-α mediated apoptosis by inhibiting the NF-kB p65 survival pathway. And then, HSP70 blocks NF-kB activation through inhibition of I-kB-α

kinase (IKK) and I- kB-α degradation. HSP70 can also facilitate DNA deletion of damaged cells (Kumar et al., 2016).

The inhibitors of growth (ING) proteins act as tumor suppressors. Their expression in cancer experiencing down regulation that transmits death signals and binds to histones and thereby controls chromatin remodeling and activity p53. This protein enhances the function of HSP70 that induces TNF-α receptor-mediated apoptosis by preventing IKK activity and blocking the NF-kB survival pathway (Kumar et al., 2016). Furthermore, cells that have hypoxia could cause imbalance of ROS and anti-ROS, where ROS will experience an increase that causes oxidative stress. It will cause any damage in protein or lipid which is used to protect itself from cell death. In this condition, tumor cells will secrete intracellular HSP70 to relieve ROS effect so that it could preserve the survival of tumor cells (Sudiana, 2011).

The emergence of HSP70 protection from dead cell necrotic mediation which resulted to JNK inhibition, does not need activity from its chaperone. HSP70 acts as DAMP and it could cause powerful immunogenic response which chronic exposure cause immune tolerance. It can be concluded that HSP70 plays multiple role in necrosis cell in malignant cells. It can also cause anti-tumor response. HSP70 also protects cancer cells from necrotic dead cell and allows apparent tumor development to be one anti-cancer treatment long period accompanied with release signal sustained necrotic disease, tumor growth and survival cells (Albakova et al, 2020).

Different results in Sherman and Gabai study stated that there was negative, correlation between HSP70 and NF-kB, HSP70 can suppress inflammation and inhibit NF-kB pathway. HSP70 in cancer with component inflammation is very important for progress because enhancement HSP70 levels will promote cancer. Tests on mice with carcinoma colon caused by the carcinogen dextran sodium sulfate accompanied with inflammation severe, knockout on HSP70 increases cytokines inflammation and exacerbation cancer (Sherman and Gabai, 2015). Even though protection from the microenvironment condition in tumor considered as a main reason for HSP70 expression excess in tumors at first, the startling observation was done by group Jaattela that HSP70 levels enhancement in cell cancer without stress required for growth even in normal conditions. Observations enhance more in carrying several cancer cells lines on and now set up different tumor cells with normal cells, where tumor cells require HSP70 for cells survival and growth (Gao et al., 2021).

The study by Albakova stated that HSP70 is a chaperone molecule expressed in the tumor cells cytoplasm. These HSP70 membrane positive in tumor cells actively release HSP70 surface positive exosomes that trigger NK cell stimulation. There were two types of HSP 70 circulating in the serum of cancer patients. One type is exosomal HSP70 released by viable tumor cells, while the other HSP70 is released by dying cancer cells

functions as damage-associated molecular patterns (DAMPs). HSP70 acts as a DAMP released by necrotic cells so that HSP70 has great immunogenic potential to elicit a strong anti-tumor T cell response either bound to tumor antigen or free antigen. HSP70 stimulates immune response, which is natural and adaptive. Extracellular HSP70 (eHSP70) is activated by regulatory T cells results in down regulation of interferon-ɣ and tumor necrosis factor-α (TNF-α) and up regulation of IL-10 and the cytokine transforming growth factor-β (TGF-). eHSP70 interacts with antigen-presenting cells (APCs) causing stimulation from cytokine inflammation such as TNF-α, IL-β, IL-6 and IL-12 through activation of nuclear factor kappa B (NF-kB) (Albakova et al, 2020).

**Conclusion**

There was no significant difference of NF-κB p65 expression in macrophages in various T stages of colorectal adenocarcinoma. There was a significant difference of HSP70 expression in tumor cells in various T stages of colorectal adenocarcinoma. The highest HSP70 expression was in T4 stage of tumour cells. There was no significant correlation between NF-κB p65 expression in macrophages and HSP70 expression in tumor cells in various T stages of colorectal adenocarcinoma

**Conflict of Interest**

The authors declare that they have no conflict of interest.

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