The effect of kefir polysaccharide extract on the expression of matrix metalloproteinase 9 (MMP-9) and integrin activation of HeLa cervical carcinoma culture cell

Yahya Irwanto1*, Eddy Mustofa1, Teguh Wiyono2, Dodi Tri Oktafianto1, Arif Rahman Nurdianto3, Robby Rinaldi Widodo4

ABSTRACT

Background: Cervical cancer is the leading cause of cancer in the world, especially in developing countries. Kefir is a goat's milk product that contains a lot of nutrients and bioactive complex which are involved in the synthesis of the anticancer, and have anticarcinogenic and antimutagenic effect. Integins are involved in various biological processes associated with cancer development, such as migration, invasion, differentiation and proliferation. MMP-9 can also degrade ECM through proteolytic action, affecting cell-cell interactions in ECM. This research aimed to see the effect of kefir on MMP-9 and integrins activation. Thus, it is expected that kefir can be used as an additional supplement in patients with cervical cancer.

Methods: This research study was conducted in an experimental laboratory in vitro using HeLa cells as the object of research. Data were analyzed using the SPSS program to determine the most effective dose of kefir and a regression test to determine the effect between integrins and MMP-9.

Results: The mean value of MMP-9 and integrin at a dose of 180 µg/ml was 165.1 ± 5.8 and 378.5 ± 12.6 respectively. This result had a significant value of p = 0.0000 for both MMP-9 and integrin-b. In the regression test, the value of p = 0.05 was obtained while the coefficient value was positive (0.565).

Conclusion: The higher the expression of MMP-9, the activation of the integrin-b would increase. Kefir extract dosage of 180 µg/ml significantly reduced MMP-9 and integrin-b expression. MMP-9 affects improving Integrin-β3

Keywords: HeLa Cells, Integrin, Kefir, MMP-9.

INTRODUCTION

In the last 10 years, cervical cancer has been the leading cause of cancer globally. In 42 developing countries, cervical cancer is the most common type. Based on the Globocan report in 2018, cervical cancer incidences were 23.4 per 100,000 populations, with a death rate of 13.9 per 100,000 populations. Carcinogenesis is a gradual process that causes cell changes from the normal stage to the pre-cancer location and finally to the early stage of cancer. Cells infected with HPV will cause abnormal cell metabolism, resulting in changes in normal cells to abnormal cells. Chronic inflammation and persistent infection have been identified as significant risk factors for cervical cancer initiation. Cervical cancer also causes an increase in the invasion and migration ability of these cells.

The pathways that drive migration and invasion of cells are the IkB kinase (IKK) pathway and the activation of the cytokine Matrix Metalloproteinase-9 (MMP-9). This pathway is also helpful as a transcription factor and mediates the motility of a cell. This activation is also related to tumour progression and aggressiveness. Integrin is a receptor located on the cell surface responsible for extracellular matrix interactions. These receptors are formed by a and b chains. Integrins are involved in various biological processes associated with cancer development, such as migration, invasion, differentiation and proliferation.

Kefir is a goat’s milk product that contains a lot of nutrients. The components of the kefir bioactive complex are involved in the synthesis of the anticancer. Besides those substances, kefir also contains many vitamins and macro elements beneficial for the body as an anticarcinogenic and antimutagenic substance. MMP is an endopeptidase that plays a vital role in physiological processes such as organogenesis, apoptosis, and cell proliferation. Disruption of MMP regulation causes tissue damage, allows motility of cancer cells and causes cell spread from the primary tumour to remote locations. MMP-9 is a protease that has the potential for various biological activities, including wound healing. MMP-9 can also degrade extracellular matrix (ECM) through proteolytic action, influencing cell-cell interactions in ECM, breaking down the extracellular domains of cell surface proteins and releasing them into the extracellular. The research that researchers want to develop is the role of kefir on MMP-9 and integrin activation...
in cervical cancer. Thus, it is expected that kefir can be used as an additional supplement in cervical cancer patients.

This research aimed to investigate the effect of kefir on MMP-9 and integrin activation. Therefore, it is expected that kefir can be used as an additional supplement in cervical cancer patients.

MATERIAL AND METHODS
Study design and preparation of samples
This research study was an experimental laboratory in vitro. This research used HeLa cells as the object of research. Samples were divided into 7 treatment groups as follows: negative control group, goat milk treatment group with a dose of 30 µg/ml, kefir treatment group with a dose of 30 µg/ml, treatment group with kefir 60 µg/ml, treatment group kefir with a dose of 120 µg/ml, group kefir treatment with a dose of 180 µg/ml and the treatment group of kefir with a dose of 240 µg/ml.

Measurement method
To measure the expression of MMP-9 and integrin-β activation by the elisa method.

Data analysis
Data were analyzed using the SPSS program using the one-way ANOVA method to determine the most effective kefir dose and regression tests to determine the effect between integrins and MMP-9.

RESULTS
Table 1 shows that the kefir dose of 180µg/ml had the lowest mean of MMP-9 and integrin-β values. The mean value of MMP-9 at a dose of 180 µg/ml was 165.1 ± 5.8, and the mean value of Integrin-β was 378.5 ± 12.6. This result had a value of p = 0.0000 MMP-9 and p = 0.0000 integrin-β. Thus, it can be concluded that the effective dose of kefir was 180 µg/ml, this was significantly different from the other groups.

A regression test was performed to test the effect of the expression MMP = 0 on integrin-β activation. In the regression test, the value of p = 0.05 was obtained while the coefficient value was positive 0.565. It means that the higher the expression of MMP-9, the activation of integrin-β would increase significantly by 56.5%.

DISCUSSION
The vital role of MMP-9 in the carcinogenesis process is its ability to support cellular invasion and tumour metastasis. MMP-9 breaks down type IV collagen as the main component of the basement membrane. MMP-9 also supports tumour invasion and angiogenesis mediated by the release of TGF-beta and VEGF. VEGF expression is also regulated by hypoxia-inducible factor-1 (HIF-1α). The proliferating tumour cells increase oxygen consumption during tumour development, thereby exacerbating hypoxia. To adapt to hypoxic conditions, tumour cells regulate HIF-1α expression, which further assists tumour development and angiogenesis. Cervical cancer is composed of malignant epithelial cells and stromal tumours consisting of extracellular matrix vascularization, fibroblasts and infiltration of inflammatory cells. Fibroblasts secrete MMP-1, MMP-2, MMP-3, MMP-10, MMP-13 and MMP-14, which play a role in tumour cell growth. Meanwhile, inflammatory cells secrete MMP-9, which induces the release of VEGF. It supports the angiogenesis process in tumours.

The research found a significant decrease in MMP-9 levels (p = 0.0000) at a 180 µg/ml dose. This research was supported by Elahe et al., 2017, Cell-free supernatants (CSF) from L casei and L. rhamnosus were used to prevent colon cancer. This research found that probiotic CSF could reduce the invasion of colon cancer cells, as evidenced by a decrease in matrix metalloproteinase-9 (MMP-9). L.rhamnosus and L. crispatus from probiotics can also decrease MMP-2 and MMP-9 expression in HeLa cells. On the other hand, the TIMP-1 pathway increases with kefir administration, TIMP-1 acts as an MMPs inhibitor. Hence, in the future. It will inhibit MMP-2 and suppress metastasis.

In a research conducted by Katia et al., kefir is known to suppress cell division and can increase apoptosis in the leukemia cell line. In this research, kefir affected MMP-9, evidenced by a decrease in MMP-9 expression. The administration of kefir at a dose of 120 µg/ml and 180 µg/ml could reduce MMP-9 levels in the cell line HeLa model of cervical cancer (p = 0.0000). Katia’s research is supported by research by Khoury et al., in which kefir can be used as an anti-proliferation and pro-apoptosis in colon cancer.

Some of the results presented show that the disturbance and dysregulation of the extracellular matrix (ECM) was the cause of cancer development. In chronic disease progression, dysregulation and remodelling of ECM and MMP will affect the growth of cancer cells, such as invasion ability, metastasis and angiogenesis (Wang et al., 2010). MMP-9 is one of the essential keys in MMP, avascular formation in tumours by stimulating VEGF. TIMP inhibitors are endogenous inhibitors that
regulate MMP-9 expression in cancer cells. MMP-9 and TIMP are endogenous and essential in reducing angiogenesis in cancer cells. On the other hand, the increase in TIMP, such as TIMP-1 and TIMP 2 in the cancer cell microenvironment, can kill cancer cells and reduce cell proliferation. 6

Integrin is a vital adhesion molecule in tumour growth, differentiation and metastasis. Integrin β1 is an integrin subunit involved in various physiological and pathological processes. Integrin β1 regulates cell growth and differentiation that supports cellular migration and proliferation. Integrin β1 can induce the expression of tumour-associated genes that play a role in tumour invasion and metastasis. β1 integrin expression is increased in cervical SCC compared to normal cervical tissue. The overexpression of β1 integrin promotes the growth of cervical epithelial cells in tumour development. Integrin has a strong association with cervical carcinoma. 10 The increase in β1 integrin bonds to its ligands in the extracellular matrix can increase cell signalling pathways, including p53 and EGF, and other signalling pathways. This also supports abnormal cell proliferation and affects the control of cell growth and differentiation, consequently leading to tumour growth. 11 Integrin is activated by several signalling intermediates (Focal Adhesion Kinase [FAK], Src, mitogen-activated protein kinase, extracellular signalling-regulated protein kinase [ERK], mammalian target of rapamycin [mTOR]). 12,13

There was a significant decrease in integrins (p = 0.000) in this research at a kefir dose of 180 µg/ml. These results were supported by research conducted by Hiroaki Konishi et al. The results showed that probiotics inhibited integrins in colon cancer through the JNK pathway. Probiotics were also reported to inhibit integrins significantly (p <0.005) at a dose of 100 µg/ml through the MAPK pathway. 14 Lunasin explained that kefir could be anti-inflammatory by inhibiting the production of IL-6 and IL-1, inhibiting NF-Akt. Integrin and COX-2. Kefir can also be used as an anticancer by inhibiting metastasis by binding directly to integrins. This binding results in the suppression of FAK / ERK / NF-κB focal signal adhesion and potentially prevents the effects of oxaliplatin. 15

As mentioned in various literature, integrins regulate the formation of adhesions in cells, participating in cell migration and the process of invasion. However, the presence of kefir shows a significant reduction of Y39-FAK after degradation of the integrin. Unlike the state of the integrin that was not given therapy, this would result in the formation of adhesions in cells, thus identifying the increase in cell size and the number of adhesions. In addition, the process of migration shows overexpression of FAK that causes disruptive effects of integrins on healthy cell migration. Conversely, if FAK is inhibited, it will hinder the up-regulatory development of the integrin. Collectively these data suggest that integrins regulate adhesion formation and promote migration and invasion of cervical carcinoma cells through FAK activity. The inhibition of integrins through the FAK pathway can inhibit the process of migration, invasion and proliferation of cervical carcinoma cells.

It is known that MMP-9 and Integrin-b are very influential in the process of cervical cancer. These two variables highly affect the angiogenesis process in cervical cancer. Qiqihar showed that 40 samples with cervical cancer are divided into high and low integrin expression, high integrin expression indicates microvascular density. The correlation test showed that integrin-b is associated with angiogenesis. This was evidenced by the HUVEC tube to confirm the involvement of integrins in the angiogenesis process. The MMP-9 enzyme decreased. This condition was in line with the decrease in FAK. This situation proved that MMP-9 and FAK are directly proportional to where FAK decreases, MMP-9 will decrease. This FAK inhibition is related to inhibition by probiotics. In which Integrin is inhibited will cause a decrease in FAK. Thus, there is a decrease in MMP-9. Collectively, these data suggested that integrins are associated with cell density and induce angiogenesis by secreting MMP-9. This result is consistent with a study conducted in which MMP-9 is significantly associated with integrins (p = 0.015). From existing research data, integrins have been shown to play an essential role in regulating MMP-9 expression in squamous epithelial cells in breast carcinoma cells. 16,17

On the other hand, integrins are also needed to accumulate MMP-9, then there will be an induction of MEK/ERK-mediated by the MMP-9 gene. This situation shows that integrin reflects the development of a malignant tumour. This evidence is also illustrated in the growth of embryonic cells or wound healing, where integrins play a role in the tissue remodelling process. 18 The function of the integrin can also control MMP-9 expression at the site of adhesion between cells because recent studies have shown that integrins are a functional component of the epithelial cell E-cadherin. 19 The conclusion is the importance of integrin function on ECM cell adhesion and cell adhesion in regulating MMP-9.

CONCLUSION
The effective dose of 180µg/ml kefir extract effectively reduces the expression of MMP-9 and Integrin-b. Increasing MMP-9 affects increasing Integrin-b.

ETHICAL CLEARANCE
This study has been declared ethically feasible by Health Research Ethics Commission of Dr. Saiful Anwar Malang Regional General Hospital Number 400/013/K.3/302/2021.

ACKNOWLEDGMENTS
Thanks to all author for participating in this study

CONFLICT OF INTEREST
All of authors reports no conflicts of interest in this manuscript.

FUNDING
This study was self-funded

AUTHOR CONTRIBUTION
All authors contributed equally in the writing of this article
REFERENCES


