3. Menguji Doktor

KODE K11

DESKRIPSI	:	Penyanggah Ujian Doktor Terbuka A.n Satuman, S.Si., M.Kes.	Halaman
BUKTI	:	Undangan ……..	02
		SK Dekan FK No 11/ UN3.1.1/HK/2021, tanggal 19 Januari 2021 …….	03
		Bukti kinerja yaitu hal sampul, hal pengesahan dll ……..	06

KEMENTERIAN PENDIDIKAN DAN KEBUDAYAAN
UNIVERSITAS AIRLANGGA
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Kampus A Jl. Mayjen Prof. Dr. Moestopo 47 Surabaya 60131
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Nomor : 177/UN3.1.1/DL/2021 10 Januari 2021
Lampiran :
Hal : Penyanggah Ujian Akhir Tahap 2 (Terbuka)

Kepada Yth,
Dr. Gondo Mastutik, drh., M.Kes
ditempat

Dengan hormat,

Dengan ini kami mengharap kehadiran Saudara sebagai **Penyanggah** Ujian Akhir Tahap 2 (Terbuka) Prodi Ilmu Kedokteran Jenjang Doktor atas nama **Satuman, S.Si., M.Kes** yang akan diselenggarakan pada :

Hari, tanggal : Selasa, 19 Januari 2021
Pukul : 10.00 – 12.00 WIB
Tempat : Aplikasi Zoom

Demikian untuk diketahui dan atas perhatian Saudara kami sampaikan terima kasih.

a.n Dekan
Wakil Dekan I,

Dr. Achmad C. Romdhoni, dr., Sp.THT-KL(K)., FICS
NIP. 197609022008011009

Catatan
- Dimohon hadir paling lambat 15 menit sebelumnya.
- Pakaian : Pria : Berjas dan berdasi
  Wanita : Menyesuaikan.

KEMENTERIAN PENDIDIKAN DAN KEBUDAYAAN
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SALINAN

KEPUTUSAN
DEKAN FAKULTAS KEDOKTERAN
NOMOR 11/UN3.1.1/HK/2021

TENTANG

PENYANGGAH UJIAN DOKTOR TERBUKA PROGRAM DOKTOR
PROGRAM STUDI ILMU KEDOKTERAN FAKULTAS KEDOKTERAN
ATAS NAMA SATUMAN, S.Si.,M.Kes.

DEKAN FAKULTAS KEDOKTERAN,

Menimbang :

a. bahwa ujian disertasi tahap I Jenjang Doktor telah dilaksanakan, selanjutnya mahasiswa yang dinyatakan lulus dari ujian tahap I tersebut berhak mengikuti ujian tahap II yang disebut Ujian Doktor Terbuka;

b. bahwa nama-nama Penyanggah Ujian Doktor Terbuka yang tercantum dalam lampiran Keputusan ini dinyatakan memenuhi syarat dan bersedia untuk ditetapkan sebagai penyanggah Ujian Doktor Terbuka;

c. bahwa berdasarkan pertimbangan sebagaimana dimaksud pada huruf a dan huruf b, perlu menetapkan Keputusan Dekan Fakultas Kedokteran Universitas Airlangga tentang Penyanggah Ujian Doktor Terbuka Program Doktor Program Studi Ilmu Kedokteran Fakultas Kedokteran.

Mengingat :

1. Undang-Undang Nomor 20 Tahun 2003 tentang Sistem Pendidikan Nasional (Lembaran Negara Republik Indonesia Tahun 2003 Nomor 78, Tambahan Lembaran Negara Nomor 4301);
2. Undang-Undang Republik Indonesia Nomor 14 Tahun 2005 tentang Guru dan Dosen (Lembaran Negara Republik Indonesia Nomor 157, Tambahan Lembaran Negara Nomor 4586;
3. Undang-Undang Nomor 12 Tahun 2012 tentang Pendidikan Tinggi (Lembaran Negara Republik Indonesia Tahun 2012 Nomor 158, Tambahan Lembaran Negara Nomor 5336);
4. Undang-Undang Nomor 5 Tahun 2014 tentang Aparatur Sipil Negara (Lembaran Negara Republik Indonesia Tahun 2014 Nomor 06, Tambahan Lembaran Negara Nomor 5494);

5. ...

5. Peraturan Pemerintah Republik Indonesia Nomor 57 Tahun 1954 tentang Pendirian Universitas Airlangga Di Surabaya sebagaimana telah diubah dengan Peraturan Pemerintah Nomor 3 Tahun 1955 tentang Pengubahan Peraturan Pemerintah Nomor 57 Tahun 1954. (Lembaran Negara Republik Indonesia Tahun 1954 Nomor 99 Tambahan Lembaran Negara Nomor 695 juncto Lembaran Negara Republik Indonesia Tahun 1955 Nomor 4 Tambahan Lembaran Negara Nomor 748);
6. Peraturan Pemerintah Nomor 4 Tahun 2014 tentang Penyelenggaraan Pendidikan Tinggi dan Pengelolaan Perguruan Tinggi. (Lembaran Negara Republik Indonesia Tahun 2014 Nomor 16, Tambahan Lembaran Negara Nomor 5500);
7. Peraturan Pemerintah Nomor 30 Tahun 2014 tentang Statuta Universitas Airlangga. (Lembaran Negara Republik Indonesia Tahun 2014 Nomor 100, Tambahan Lembaran Negara Nomor 5535);
8. Peraturan Rektor Universitas Airlangga Nomor 38 Tahun 2017 tentang Peraturan Pendidikan Universitas Airlangga;
9. Peraturan Rektor Universitas Airlangga Nomor 21 Tahun 2014 tentang Pedoman Pendidikan Program Doktor (S3) Universitas Airlangga;
10. Keputusan Rektor Universitas Airlangga Nomor 1947/H3/KR/2011 tentang Penetapan Ruang Lingkup Program Studi dalam Kategori Monodisiplin, Interdisiplin dan Multidisiplin untuk Pengelolaan Program Magister dan Program Doktor;
11. Keputusan Rektor Universitas Airlangga Nomor 762/UN3/KR/2020 tentang Pengangkatan Dekan Fakultas, Direktur Sekolah Pascasarjana, dan Direktur Rumah Sakit Periode 2020-2025.

MEMUTUSKAN:

Menetapkan : KEPUTUSAN DEKAN FAKULTAS KEDOKTERAN TENTANG PENETAPAN PANITIA UJIAN DISERTASI SEBAGAI UNDANGAN AKADEMIK UJIAN DOKTOR TERBUKA PROGRAM DOKTOR PROGRAM STUDI ILMU KEDOKTERAN FAKULTAS KEDOKTERAN ATAS NAMA SATUMAN, S.Si.,M.Kes.

PERTAMA: ...

PERTAMA : Menetapkan Penyanggah Ujian Doktor Terbuka Program Doktor Program Studi Ilmu Kedokteran Fakultas Kedokteran atas nama Satuman, S.Si.,M.Kes. yang dilaksanakan pada tanggal, 19 Januari 2021 dengan susunan nama sebagai berikut:

1. Prof. Dr. Eddy Bagus Wasito, dr.,MS.,Sp.MK(K)
2. Prof. Dr. Soemarno, dr.,DMM.,Sp.MK(K)
3. Prof. Dr. Widjiati, drh.,M.Si
4. Dr. Dwi Aprilawati, dr.,M.Kes.,Sp.GK
5. Dr. Dominicus Husada, dr.,DTM&H.,MCTM(TP).,Sp.A(K)
6. Dr. Anggraini Dwi Sensusiati, dr.,Sp.Rad(K)
7. Dr. Gondo Mastutik, drh,.M.Kes.
8. Dr. Rochmah Kurnijasanti, drh.,M.Si
9. Prof. Dr. I Ketut Sudiana, Drs.,M.Si
10. Prof. Dr. Budi Santoso, dr., Sp.OG(K)

KEDUA : Dalam menjalankan tugasnya sebagaimana dimaksud dalam diktum PERTAMA, berpedoman pada peraturan dan ketentuan yang berlaku serta mempertanggungjawabkan tugasnya kepada Dekan Fakultas Kedokteran.

KETIGA : Biaya untuk keperluan tersebut dibebankan pada dana Rencana Kegiatan dan Anggaran Tahunan (RKAT) Fakultas Kedokteran.

KEEMPAT : Keputusan ini mulai berlaku pada tanggal ditetapkan.

Ditetapkan di Surabaya
pada tanggal 19 Januari 2021

DEKAN,

ttd

Budi Santoso
NIP. 196302171989111001

Salinan sesuai dengan aslinya
Kepala Bagian Tata Usaha,

Basuni
NIP. 196501021987011001

SALINAN disampaikan Yth.
1. Rektor Universitas Airlangga
2. Yang bersangkutan

Diterbitkan Untuk Ujian Akhir Tahap 2 (Terbuka)

DISERTASI

MEKANISME STIMULASI KANKER OLEH PROTEIN EFEKTOR AvrA *Salmonella typhimurium* PADA KANKER KOLON

SATUMAN

PROGRAM STUDI ILMU KEDOKTERAN JENJANG DOKTOR
FAKULTAS KEDOKTERAN UNIVERSITAS AIRLANGGA
SURABAYA
2021

MEKANISME STIMULASI KANKER OLEH PROTEIN EFEKTOR AvrA *Salmonella typhimurium* PADA KANKER KOLON

DISERTASI

Untuk memperoleh Gelar Doktor dalam Program Studi Ilmu Kedokteran Jenjang Doktor pada Fakultas Kedokteran Universitas Airlangga dan dipertahankan di hadapan Panitia Ujian Akhir Tahap 2 (Terbuka)

Oleh;
SATUMAN
011717017325

PROGRAM STUDI S3 ILMU KEDOKTERAN JENJANG DOKTOR
FAKULTAS KEDOKTERAN UNIVERSITAS AIRLANGGA
SURABAYA
2021

LEMBAR PENGESAHAN

DISERTASI

MEKANISME STIMULASI KANKER OLEH PROTEIN EFEKTOR AvrA *Salmonella typhimurium* PADA KANKER KOLON

TELAH DISETUJUI

PADA TANGGAL 23 DESEMBER 2020

Oleh

Promotor

Prof. Dr. Eddy Bagus Wasito, dr. MS, SpMK(K)
NIP. 19510221 1978021 001

Ko promotor

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NIP. 19480706 1980021 001

Mengetahui

Ketua Program Studi Ilmu Kedokteran Jenjang Doktor
Fakultas Kedokteran Universitas Airlangga

Prof. Dr. H. Hendy Hendarto, dr., Sp. OG(K)
NIP. 196108172016016101

Disertasi ini telah disetujui untuk diuji dan dinilai
oleh panitia penguji Ujian Tahap 1 (Tertutup)
pada Tanggal 04 Desember 2020

Panitia Penguji:

Ketua : 1. Prof. Dr. Drs. I Ketut Sudiana, M. Si

Anggota : 2. Prof. Dr. Eddy Bagus Wasito, dr. MS, SpMK(K)
3. Prof. Dr. Soemarno, dr. DMM, SpMK(K)
4. Prof. Sofia Mubarika, dr. M.Med.Sc., PhD
5. Prof. Dr. Fedik Abdul Rantam, drh
6. Dr. Eddy Herman Tanggo, dr. SpBOnk
7. Dr. Hari Basuki Notobroto, dr. M. Kes

Ditetapkan dengan Surat Keputusan
Dekan Fakultas Kedokteran Universitas Airlangga
Tentang Panitia Penguji Disertasi
Nomor: 432/UN3.1.1/HK.04/2020
Tanggal: 4 Desember 2020

SUMMARY

Colorectal cancer is the highest cause of morbidity and death in the world. In Indonesia, colorectal cancer is the highest malignancy, often occurring in both men and women, after prostate cancer and breast cancer of which percentage is 11.5% of the total number of cancer patients in Indonesia. The incidence of colorectal cancer in men is comparable to women and is more common found in the prolific age. The colorectal cancer is triggered by several factors including carcinogenic chemicals, ultraviolet light, viruses and bacteria causing cell mutations. One of the bacteria that is suspected as the risk factor for colorectal cancer is *Salmonella*. *Salmonella* is a genus of Gram-negative bacteria in the group of enterobacteriaceae with the highest pathogenicity which can elicit cross infections between humans and animals. Until recently the relationship between infections with the occurrence of colorectal cancer remains a mystery. One of the effectors playing an important role in the infection is AvrA. AvrA effector protein is an acetyltransferase group of which molecular weight is 33-34 kDa expressed in several enteric pathogens. The mechanism of AvrA effector in the pathogenesis of Salmonellosis has not been unrevealed yet. The pathogenesis of *Salmonellosis* involves 4 (four) processes ranging from the bacteria attachment to the intestinal lumen, bacteria multiplication in the Peyer's patch macrophage, and bacteria survival in the bloodstream further producing enterotoxins causing electrolytes and water to discharge into the intestinal lumen. The early stage of *Salmonella* infection is characterized by phagocyte activation by macrophages, inflammation of infected tissue and production of gamma Interferon (IFN) by several cells. $CD4^+$ T cells, besides $CD8^+$ and $T\gamma\delta$ cells, play an important role in controlling *S. typhimurium* infections. Inflammation and uncontrolled ROS for host cells can ultimately damage the tissue occupied by *Salmonella* during the infection. The role of ROS in the stem cell microenvironment is still not widely known however it is presumed that ROS can possibly cause stem cell DNA damage. Immune cell activation, increased inflammation and ROS are microenvironments which are able to transform the stem cells into cancer stem cells. However, this mechanism is still unknown. Therefore, this study was conducted to analyze the mechanism of *S. typhimurium* and AvrA infections against blood lymphocyte T cell stimulation, the increase of intracellular reactive oxygen species (ROS), the increase of IFN-γ levels, the number of stem cell cancer populations, the decrease of PTEN expression and the increase of c-Myc tissue expression in mice model of the colorectal cancer infected with *S. typhimurium*.

The study was conducted *in vivo* on mice treated with AvrA and *S. typhimurium*. The AvrA administration and *S. typhimurium* infection were conducted together with the provision of azoxymethane (AOM) as an adjuvant to accelerate the occurrence of the colorectal cancer. This administration was conducted in series of time, 1 week and 12 weeks. The parameters observed were the expression of AvrA in the colon tissue with immunohistochemistry method, the number of regulator T cells and intracellular ROS with the flow cytometry method, IFN-γ levels with the ELISA method, the number of cancer stem cells (CCSC) with the flow

cytometry method, the expression of PTEN and c-Myc in the tissue colon with immunohistochemistry method.

The study's result showed that AvrA tended to have more binding with CXCR3 compared to TLR4 or SPI-1. The result of the analysis on the colon tissue expression of AvrA in the week-1 increased in the group after AvrA (30.37±4.31) and infection *S. typhimurium* (23.06±0.71) compared to the control group (7.11±7,48) and the administration of AOM only (7.02±4.18). There was no significance on the control group (13.81±2,30) and AOM (16.39±1.75) concerning the expression of AvrA in the week-12. The significance of the increase of the AvrA expression was obtained on the group of AOM+AvrA (78.29±10.45). On the other hand, significant decrease occurred on the group of AOM+*S. typhimurium* (47.63±1.49). In the week-1 a significant change occurred on AOM group (0.03±0.04) on the T cell regulator. Furthermore, the significance of the increase of the T regulator expression was gained on AOM+*S. typhimurium* (0.17±0.07). In the week-12, there was significant difference on AOM+*S. typhimurium* (0.17 ± 0.07), and AOM (2.59 ± 2.77) group of the T regulator cell. The level of IFN-γ increased in the week-12 and the increase occurred on AOM, AOM+AvrA and AOM+*S. typhimurium* groups compared to the control group. In the week-1 of induction it could be observed that the level of IFN-γ tended to increase on the groups of AOM (348.75±69.91), AOM+AvrA (698.00±232.04) and AOM+*S. typhimurium* (547.75±312.12) compared to the control (500.75±129.79) although there was no difference on each of the groups respectively. In the week-12 it was found that the content of IFN-γ increased due to induction of AOM (1598.50±856.94), AOM+AvrA (1393.00±307.26) and AOM+*S. typhimurium* (1728.17±862.99) compared to the control (346±268.29). In the week-1 of induction it could be explained that the total of intracellular ROS tended to increase on AOM (23.32 ± 1.87), AOM+AvrA (21.19±8.3) and AOM+*S. typhimurium* (23.12±2.86) compared to the control (13.22±8.52). It was found in the week-12 that the level of ROS increased due to AOM+*S. typhimurium* (45.78±2.93) induction compared to AOM (3.51±0.61), AOM+AvrA (24.76±4.71) and the control (4.86±2.30) groups. In the week-1 of CCSC there was significant change on the control (0.03±0.02) and AOM (022±0.10) groups. The significance of expression increase of CCSC was obtained on AOM+AvrA (0.60±10.45) groups on the other hand the AOM+*S. typhimurium* (0.44±0.13) group underwent significant decrease. In the week-12 of CCSC there was significant difference between the control (0.84 ± 1.15), and the AOM+AvrA (3.17 ± 1.05) groups. Besides it was found that there was significant difference between AOM (2.15 ± 0.81) and AOM+ *S typhimurium* (0.51 ± 0.29). In the week-1 of PTEN there was significance of change on the control (22.98 ± 8.57), AOM (85.52 ± 5.43), AOM+AvrA (52.42±7.59) and AOM + *S. typhimurium* (38.88 ± 2.54) groups. The significance of the expression increase of PTEN in the week-12 was obtained on the control (26.75 ± 7.73), AOM (74.62 ± 8.78), AOM+AvrA (62.48 ± 4.03) and AOM + *S. typhimurium* (49.53 ± 3.98) groups. Moreover, there was significance of change in the week-1 of c-Myc on the control (35.12 ± 3.31), AOM (51.21 ± 11.41), AOM+AvrA (83.25 ±

4.08), and AOM + *S. typhimurium* (70.35 ± 9.61) groups. The significance of the expression increase of c-Myc in the week-12 was obtained on the control (19.05 ± 4.41), AOM (50.59 ± 6.37), AOM+AvrA (75.67 ± 13.53), and AOM + *S. typhimurium* (75.96 ± 6.32) groups.

It can conclude based on the study that AvrA expression increased in the week-1 and 12 in mice model of colorectal cancer. Regulator T cell expression significantly increased in the week-1 and and it significantly increased in week-12 after it had been given AOM + AvrA. The expression IFN-γ increased in the week-1 in mice model of colorectal cancer after being given AvrA and *S. typhimurium* infections for 12 weeks. Furthermore, the number of ROS tended to increase after the administration of AOM conducted on the week-1 and it increased on the week 12 after the administration of AOM + *S. typhimurium*. Increased CCSC number was obtained on the AOM + AvrA group in the week-1 and 12. Besides, PTEN expression increased in the week-1 and 12 and it was found that there was significant difference after the administration of AOM. c-Myc expression increased in the week-1 and 12 and it was found that there was significant difference after AOM + AvrA administration.

ABSTRACT

Background: One of the risk factors for colorectal cancer is *Salmonella* bacterial infection. *Salmonella typhimurium* is a Gram-negative bacterium that is able to trigger cross infection between humans and animals. This bacterium possibly becomes the risk factor for colorectal cancer. *Salmonella* and its effector, AvrA, are able to convert stem cells into cancer stem cells through changes in the microenvironment by suppressing the immune system, increasing inflammation and radical oxygen species (ROS). AvrA effector may be able to disrupt PTEN signals and regulatory T cells and affect c-Myc as a proto-oncogene of malignancy.

Purpose: The aim of this study was to explain the mechanism of AvrA and *Salmonella typhimurium* infection as risk factors for colorectal cancer by observing AvrA bonds in the colon, AvrA expression, activation of regulator T cells, IFN-γ level, the number of intracellular ROS and cancer stem cells, PTEN expression and its c-myc in mice model of colorectal cancer induced with axozymethane.

Methods: This was a factorial experimental research. This research used Balb/c mice model of colorectal cancer with azoxymethane induction and they were given AvrA and infected with *Salmonella typhimurium in vivo*. The AvrA was isolated from *Salmonella typhimurium* protein. Testing of AvrA bonds with receptors or chemokines was done by docking using CLUSPRO 2.2 software. The amount of forty mice were divided into several groups which were control group, group given azoxymethane (AOM) only, group given AOM + AvrA, and group given AOM + *S. typhimurium*. The mice were killed after 1st week and 12th weeks of treatment. AvrA, PTEN and c-Myc expressions were analysed with immunohistochemistry method, regulator T cells, ROS and the colon cancer stem cell numbers were analysed with flow cytometry method, and IFN-γ levels were analysed with ELISA method. The Statistical analysis were analysed with *Kruskal Wallis* and *Mann Whitney* and followed by Path analysis.

Results: AvrA *Salmonella typhimurium* penetrated the colon cells by binding to CXCR3. This study found significant differences in AvrA expression, regulatory T cells, IFN-γ levels, intracellular ROS amount, colon cancer stem cells, PTEN expression, c-Myc expression in each group in 1st week and 12th weeks.

Conclusion: AvrA *Salmonella typhimurium* was able to induce colorectal cancer.

Keywords: *acute inflammation, chronic inflammation, AvrA, Salmonella typhimurium, colorectal cancer*