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EFFECTS OF TRICLOSAN ON ANOIKIS-RESISTANT HUMAN NON-SMALL CELL LUNG
CANCER H460 CELLS

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A Dissertation Submitted in Partial Fulfillment of the Requirements
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ธิดารัตน์ วิจิตรนา : ผลของไตรโคลซานต่อเซลล์มะเร็งปอดมนุษย์ชนิดไม่ใช่เซลล์เล็ก
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การเปลี่ยนแปลงลักษณะเซลล์จากอีพิธิเลียลเป็นเซลล์มีเซ็นโคมอล (epithelial-to-mesenchymal transition; EMT) เป็นสาเหตุหนึ่งที่ทำให้เซลล์มะเร็งมีความรุนแรงเพิ่มมากขึ้น
เนื่องจากมีผลเพิ่มการแพร่กระจายของเซลล์มะเร็ง งานวิจัยนี้เป็นการรายงานถึงผลของไตรโคล
ซาน ซึ่งใช้เป็นสารฆ่าเชื้อแบคทีเรียในผลิตภัณฑ์ต่างๆ ต่อการเหนี่ยวนำให้เกิดกระบวนการ EMT
ในเซลล์มะเร็งปอดของมนุษย์ที่ต่อต้านการตายแบบอะนอยคิส โดยกระบวนการ EMT เป็น
กระบวนการที่ทำให้เซลล์มะเร็งเพิ่มความสามารถในการเคลื่อนที่และการรุกราน รวมถึงเพิ่ม
ความสามารถในการสร้างมะเร็งก้อนใหม่อีกด้วย ในงานวิจัยนี้เซลล์มะเร็งที่ต่อต้านการตายแบบอะ
นอยคิสได้รับไตรโคลซานในความเข้มข้นที่สัมพันธ์กับระดับของสารที่พบในร่างกายมนุษย์ จาก
การศึกษาพบว่าไตรโคลซานมีผลทำให้เซลล์มะเร็งลดการเกาะกันระหว่างเซลล์ซึ่งเป็นลักษณะ
สำคัญของเซลล์ที่ผ่านกระบวนการ EMT และเมื่อศึกษากลไกการออกฤทธิ์ของไตรโคลซานด้วย
วิธีการ western blot analysis พบว่า ไตรโคลซานมีผลทำให้ระดับของ E-cadherin ในเซลล์
ลดลงอย่างมีนัยสำคัญทางสถิติ และเพิ่มการแสดงออกของ EMT markers ได้แก่ N-cadherin,
vimentin, snail และ slug อย่างมีนัยสำคัญทางสถิติอีกด้วย ซึ่งแสดงให้เห็นว่าเซลล์มะเร็งที่ได้รับ
ไตรโคลซานมีลักษณะเป็นมีเซ็นโคมอลเพิ่มมากขึ้น และเมื่อศึกษาด้วยวิธีการ tumor formation
assay พบว่าการเหนี่ยวนำให้เกิด EMT ด้วยไตรโคลซานยังมีผลเพิ่มความสามารถในการสร้าง
โคโลนีของเซลล์มะเร็งอย่างมีนัยสำคัญทางสถิติ นอกจากนี้การเหนี่ยวนำให้เกิด EMT ด้วยไตรโคล
ซานยังมีผลกระตุ้นการทำงานของ focal adhesion kinase/ATP dependent tyrosine
kinase (FAK/Akt) และ Ras-related C3 botulinum toxin substrate 1 (Rac1) จึงทำให้
เซลล์มะเร็งมีความสามารถในการเคลื่อนที่และการรุกรานเพิ่มมากขึ้น ดังนั้นจึงกล่าวโดยสรุปได้ว่า
การศึกษานี้เป็นการรายงานถึงผลการเหนี่ยวนำของไตรโคลซานให้เกิด EMT ในเซลล์มะเร็งปอด
ของมนุษย์ที่ต่อต้านการตายแบบอะนอยคิส ซึ่งมีผลเพิ่มความสามารถในการสร้างโคโลนีของ
เซลล์มะเร็งและความสามารถในการเคลื่อนที่ของเซลล์มะเร็ง โดยคุณสมบัติที่ได้กล่าวมานี้เป็น
ปัจจัยสำคัญที่มีผลทำให้เซลล์มะเร็งเกิดการแพร่กระจายได้มากยิ่งขึ้น ผลการศึกษานี้ทำให้ได้
ข้อมูลใหม่ด้านพิษวิทยาของไตรโคลซาน โดยเฉพาะอย่างยิ่งข้อมูลประกอบการพิจารณาใช้สารนี้
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THIDARAT WINITTHANA: EFFECTS OF TRICLOSAN ON ANOIKIS-RESISTANT HUMAN NON-SMALL CELL LUNG CANCER H460 CELLS. ADVISOR: ASST. PROF. PITHI CHANVORACHOTE, Ph.D., CO-ADVISOR: ASSOC. PROF. POL. LT. COL. SOMSONG LAWANPRASERT, Ph.D., 124 pp.

Alteration of epithelial cancer cell toward mesenchymal phenotype (epithelial-to-mesenchymal transition; EMT) has been shown to potentiate tumor aggressiveness by increasing cancer cell metastasis. Herein, the present study demonstrates the effect of triclosan, a widely used antibacterial agent found in many daily products, in enhancing the EMT in aggressive anoikis resistant human lung cancer cells. EMT was long known to increase abilities of cancer cells to increase migration and invasion as well as tumorigenicity of cells. The present study reveals that treatment of the anoikis resistant cells with triclosan at the physiologically-related concentrations significantly decreased cell to cell adhesion which is a dominant characteristic of cell undergoing EMT. Importantly, western blot analysis revealed that triclosan-treated cells exhibited decreased E-cadherin, while the levels of EMT markers, namely N-cadherin, vimentin, snail and slug were found to be significantly up-regulated, indicating mesenchymal phenotype of cells. Also, EMT-induced by triclosan treatment increased the colony number of the cancer cells assessed by tumor formation assay. Furthermore, EMT-induced by triclosan treatment was accompanied by the activation of focal adhesion kinase/ATP dependent tyrosine kinase (FAK/Akt) and Ras-related C3 botulinum toxin substrate 1 (Rac1) which enhanced ability of the cells to migrate and invade. In conclusion, this study demonstrated that triclosan may potentiate tumorigenicity and motility of anoikis resistant human lung cancer cells via the process of EMT. As mentioned capabilities are required for success in metastasis, the present study provides the novel toxicological information and encourages the awareness of triclosan use in cancer patients

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LIST OF ABBREVIATIONS

%	= percentage
°C	= degree Celsius
µg	= microgram (s)
µl	= microliter (s)
µM	= micromolar
Akt	= adenosine triphosphate dependent tyrosine kinase
ANOVA	= analysis of variance
APAF	= apoptosis protease activating factor
AR	= androgen receptor
AR cells	= anoikis resistant cells
ATP	= adenosine triphosphate
Bak	= Bcl-2 homologous antagonist killer
Bax	= Bcl-2-associated X protein
Bcl-2	= B-cell lymphoma 2
Bcl-XL	= B-cell lymphoma-extra large
bHLH	= basic helix-loop-helix
Bid	= BH3 interacting-domain death agonist
Bim	= Bcl-2-like protein 11
BRCA2	= breast cancer 2
BSA	= bovine serum albumin
Ca ²⁺	= calcium ion
Cdc42	= cell division cycle 42
CO ₂	= carbon dioxide
CTCs	= circulating tumor cells
CR	= cytokine receptor
DFF40	= DNA fragmentation factor 40 kDa
DISC	= death-inducing signaling complex
DMSO	= dimethyl sulfoxide
DNA	= deoxyribonucleic acid
DNMT	= DNA methyltransferases



EC	= extracellular cadherin domain
ECM	= extracellular matrix
EDTA	= ethylenediaminetetraacetic acid
EGF	= epidermal growth factor
EGFR	= epidermal growth factor receptor
EMT	= epithelial-to-mesenchymal transition
EPA	= The US Environmental Protection Agency
ER	= estrogen receptors
ERB4	= receptor tyrosine-protein kinase erbB-4
ERK	= extracellular-signal-regulated kinase
et al.	= et alibi, and other
FADD	= Fas-associated death domain protein
FAK	= focal adhesion kinase
FasL	= Fas Ligand
FBS	= fetal bovine serum
FDA	= The US Food and Drug Administration
FGF	= fibroblast growth factor
g	= gram
GAPs	= GTPase activating proteins
GDI	= guanine nucleotide dissociation inhibitors
GDM	= global DNA methylation
GDP	= guanosine diphosphate
GEFs	= guanine nucleotide exchange factors
GF	= growth factor
GFR	= growth factor receptor
GFRKs	= growth factor receptor kinases
GPCR	= G-protein-coupled receptor
GTP	= guanosine triphosphate
h	= hour, hours
H ⁺	= hydrogen ion
HRP	= horseradish peroxidase



IgG	= immunoglobulin G
IU	= international unit
kDa	= kilo dalton
MAPK	= mitogen-activated protein kinase
MET	= mesenchymal-epithelial transition
MDB	= methylated DNA-binding domain
min	= minute (s)
mg	= milligram (s)
ml	= milliliter
mM	= millimolar
MMPs	= matrix metalloproteinases
mTOR	= mammalian target of rapamycin
mTORC1	= mammalian target of rapamycin complex 1
mTORC2	= mammalian target of rapamycin complex 2
MTT	= 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
Na ⁺	= sodium ion
NaCl	= sodium chloride
nm	= nanometer (s)
NSCLC	= non-small cell lung cancer
OD	= optical density
OMM	= outer mitochondrial membrane
p53	= cellular tumor antigen p53
PBS	= phosphate-buffered saline
pAkt	= phosphorylated adenosine triphosphate dependent tyrosine kinase
pFAK	= phosphorylated focal adhesion kinase
PDK1	= phosphoinositide-dependent kinase-1
PI	= propidium iodide
PI3K	= phosphatidylinositol-3 kinase
PIP2	= polyhydroxyl- phosphatidylinositol (4,5)-bisphosphate
PIP3	= phosphatidylinositol (3,4,5)-trisphosphate



PKB	= protein kinase B
PPAR α	= peroxisome proliferator-activated receptor alpha
pro-MMPs	= pro-matrix metalloproteinases
PUMA	= p53 upregulated modulator of apoptosis
Rac1	= Ras-related C3 botulinum toxin substrate 1
RhoA	= Ras homolog gene family, member A
Ras	= rat sarcoma
RPMI	= Roswell Park memorial institute's medium
SCLC	= small cell lung cancer
SDS	= sodium dodecyl sulfate
SE	= standard error
Ser	= serine
SF/HGF	= scatter factor/hepatocyte growth factor
Src	= sarcoma
T3	= triiodothyronine
T4	= thyroxine
t-Bid	= truncated form of Bid
TBST	= Tris-buffered saline, 0.1% Tween 20
TCS	= 2,4,4'-trichloro-2'-hydroxydiphenyl ether
TGF- β	= transforming growth factor beta
Tyr	= tyrosine
ZEB	= zinc finger E-box-binding homeobox
ZO	= zonula occludens

