

Nanoscale

ARTICLE

The Impact of Tomato Fruits Containing Multi-walled Carbon Nanotube Residues on Human Intestinal Epithelial Cell Barrier Function and Intestinal Microbiome Composition

Accepted 00th January 20xx

DOI: 10.1039/x0xx00000x

www.rsc.org/

Mohamed H. Lahiani^{a,b}, Sangeeta Khare^b, Carl E. Cerniglia^b, Ramiz Boy^{c,d}, Ilia Ivanov^c, and Mariya Khodakovskaya^{1*}

^{a.} Department of Biology, University of Arkansas at Little Rock, Little Rock, AR, 72204, USA e-mail: mvkhodakovsk@ualr.edu

^{b.} Division of Microbiology, National Center for Toxicological Research, FDA, Jefferson, AR, 72079.

^c Center for Nanophase Materials Sciences, Oak Ridge National Laboratory, Oak Ridge, TN, 37831, USA

^d. Department of Textile Engineering, Çorlu Faculty of Engineering, Namık Kemal University, Çorlu-Tekirdağ 59860, TURKEY

⁺ Footnotes relating to the title and/or authors should appear here.

Electronic Supplementary Information (ESI) available:Fig. S1 is Bright field microscopy of T-84 cells after exposure to CNT, Fig. S2 is shematic diagram o fNGS data analysis, Fig. S3-5 deatiled heatmap of top significantly altered genes, Table S1-3 include list of gene classification, Fig. S6 confirmation of NGS data using Real-time PCR, Fig. S7-9 PCA based on time, indivudals and doses used. See DOI: 10.1039/x00x0000x

J. Name., 2013, 00, 1-3 | 1 This journal is © The Royal Society of Chemistry 20xx

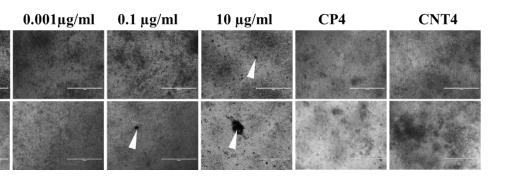


Figure S1. Bright field microscopy of T-84 cells after exposure to CNT at different doses (0.001, 0.1, and 10 μ g/ml), control fruits extract (CP4), and CNT-containing fruits (CNT4) during two-time points (1- and 48-hour post-exposures). Arrows showing the agglomeration of CNT after long time exposure. Figures were taken at 20X magnification. Scale bar equal to 100 μ m.

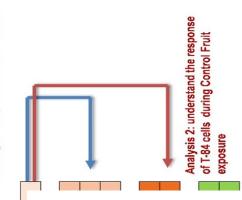


Figure S2. Schematic diagram of the 4 different analysis considered during the data analysis of RNA Next Generation Sequencing. Analysis 1 included the comparison of gene expression of T-84 cells exposed to CNT (at 3 concentrations) versus the negative (untreated) control. Analysis 2 was a comparison between transcriptome of cells exposed to control fruits versus the negative (untreated) control. Analysis 3 was made by the comparison of gene expression of cells exposed to CNT- containing fruits versus the negative (untreated) control. Analysis 4 included the comparison of transcriptome of two group of cells exposed to control fruits or to fruits containing MWCS2

Different experimental conditions

Analysis 3: understand response of T-84 cells during CNT and Fruit exposure

C1 · 1	. 1	
Classical	Ana	VCIC
Classical	1 ma	1 9 515

-	up-regulated	Down-regulated	Total
Experimental Study 1			
0.001 µg/ml CNT vs Negative Control	8	11	19
0.1 µg/ml CNT vs Negative Control	8	18	26
10 µg/ml CNT vs Negative Control	97	39	136
Experimental Study 2			
Control fruit vs Negative Control	643	414	1057
Experimental Study 3			
CNT fruit vs Negative Control	129	77	206
Experimental Study 4			
CNT fruit vs Control fruit	500	114	614

Table S1. The number of genes with significantly altered expression in control and CNT-treated T-84 cells. The analysis was performed on the base of Next Generation Sequencing data using Seralogix software. All gene numbers represent significantly perturbed genes, through either upor down-regulation (p<0.001 and fold change threshold of 0.35).



L1CAM: L1 cell adhesion molecule MX2: MX dynamin-like GTPase 2 MUC16: mucin 16, cell surface associated SAMD9: sterile alpha motif domain containing 9 SAMD9L: sterile alpha motif domain containin... XAF1: XIAP associated factor 1 UCA1: urothelial cancer associated 1 (non-pro... PARP9: poly (ADP-ribose) polymerase family, ... CMPK2: cytidine monophosphate (UMP-CMP) ... TRIM22: tripartite motif containing 22 DDX58: DEAD (Asp-Glu-Ala-Asp) box polypep... NLRC5: NLR family, CARD domain containing 5 TACSTD2: tumor-associated calcium signal tra... SLC2A3: solute carrier family 2 (facilitated gl... PRIC285: peroxisomal proliferator-activated re... FN1: fibronectin 1 PHLDB2: pleckstrin homology-like domain, fa... MX1: MX dynamin-like GTPase 1 OASL: 2'-5'-oligoadenylate synthetase-like AKAP12: A kinase (PRKA) anchor protein 12 IFI16: interferon, gamma-inducible protein 16 PODXL: podocalyxin-like SLC2A14: solute carrier family 2 (facilitated gl... IFIH1: interferon induced with helicase C dom ... EPSTI1: epithelial stromal interaction 1 (breast) IFIT1: interferon-induced protein with tetratri... IFITM3: interferon induced transmembrane p... C1orf116: chromosome 1 open reading frame ... THBS1: thrombospondin 1 PTRF: polymerase I and transcript release factor DHRS2: dehydrogenase/reductase (SDR famil... ENTHD1: ENTH domain containing 1 NHSL2: NHS-like 2 TMEM160: transmembrane protein 160 RPS26P11: ribosomal protein S26 pseudogen... SLC36A2: solute carrier family 36 (proton/ami... RPS21: ribosomal protein S21 MALAT1: metastasis associated lung adenoca... MINOS1: mitochondrial inner membrane orga... TMEM132C: transmembrane protein 132C FLJ39653: uncharacterized FLJ39653 RPLPOP2: ribosomal protein, large, PO pseudo... ANKRD30B: ankyrin repeat domain 30B DFNB31: deafness, autosomal recessive 31 RGS9: regulator of G-protein signaling 9 KIAA0825: KIAA0825 GCSH: glycine cleavage system protein H (a... ZNF141: zinc finger protein 141

Figure S3. Heat map of the top 48 significantly perturbed genes during exposure of control tomato fruits to T-84 epithelial cells. Tomato fruits extract were exposed at a concentration of $3\mu g/ml$.

Gene expression results are based on the number of RNA-seq transcripts detected, which were normalized and expressed as fold change compared to the untreated control. The fold change threshold score was selected for a 98% confidence. Darker red gradient indicates increased/higher gene expression while darker green gradient indicates suppression/lower of gene expression.

Fold Change Coding <-1.20 0.0 >1.20 BayesScore threshold:0.6

MX1: MX dynamin-like GTPase 1 OAS3: 2'-5'-oligoadenylate synthetase 3, 100.. IFIT3: interferon-induced protein with tetratri... PARP9: poly (ADP-ribose) polymerase family, ... SAMD9: sterile alpha motif domain containing 9 DMBT1: deleted in malignant brain tumors 1 IFITM3: interferon induced transmembrane p... SAMD9L: sterile alpha motif domain containin... XAF1: XIAP associated factor 1 MX2: MX dynamin-like GTPase 2 UCA1: urothelial cancer associated 1 (non-pro... DDX58: DEAD (Asp-Glu-Ala-Asp) box polypep... MSN: moesin CMPK2: cytidine monophosphate (UMP-CMP) ... SERPINB2: serpin peptidase inhibitor, clade B ... IFI27: interferon, alpha-inducible protein 27 ISG15: ISG15 ubiquitin-like modifier OASL: 2'-5'-oligoadenylate synthetase-like PTRF: polymerase I and transcript release factor IFIT2: interferon-induced protein with tetratri... ANKRD1: ankyrin repeat domain 1 (cardiac m... RSAD2: radical S-adenosyl methionine domai... IFI16: interferon, gamma-inducible protein 16 KRT17: keratin 17 ALPP: alkaline phosphatase, placental IGFBP2: insulin-like growth factor binding pro... KIAA0825: KIAA0825 SERPINA12: serpin peptidase inhibitor, clade ... C1orf83: chromosome 1 open reading frame 83 UNC45B: unc-45 homolog B (C. elegans) HFM1: HFM1, ATP-dependent DNA helicase h... ANKRD30BL: ankyrin repeat domain 30B-like

EYA4: EYA transcriptional coactivator and pho... MALAT1: metastasis associated lung adenoca... SLC36A2: solute carrier family 36 (proton/ami... NOTCH2: notch 2

DLG2: discs, large homolog 2 (Drosophila) NOTCH2NL: notch 2 N-terminal like **Figure S4.** Heat map of the top 38 significantly perturbed genes during exposure of tomato CNTcontaining fruitsto T-84 epithelial cells. Tomato fruits extracts were exposed at a concentration of 3μ g/ml. Gene expression results are based on the number of RNA-seq transcripts detected, which were normalized and expressed as fold change compared to the untreated control. The fold change threshold score was selected for a 98% confidence. Darker red gradient indicates increased/higher gene expression while darker green gradient indicates suppression/lower of gene expression. Fold Change Coding

WDR38: WD repeat domain 38 CCDC85B: coiled-coil domain containing 85B TMEM160: transmembrane protein 160 GCSH: glycine cleavage system protein H (a... DAPK1: death-associated protein kinase 1 ARL14EPL: ADP-ribosylation factor-like 14 effe... PDZK3: PDZ domain containing 3 BDNF-AS: BDNF antisense RNA SEC61G: Sec61 gamma subunit RPS21: ribosomal protein S21 MIR147B: microRNA 147b RPS27A: ribosomal protein S27a NME1: NME/NM23 nucleoside diphosphate kin... RPL39L: ribosomal protein L39-like PSENEN: presenilin enhancer gamma secreta... DFNB31: deafness, autosomal recessive 31 LINGO1: leucine rich repeat and Ig domain co... RPL21: ribosomal protein L21 RPL21P28: ribosomal protein L21 ALKBH7: alkB, alkylation repair homolog 7 (E. ... ZNF141: zinc finger protein 141 FLJ33534: uncharacterized LOC285150 MRPL41: mitochondrial ribosomal protein L41 TOMM7: translocase of outer mitochondrial m... RPLP2: ribosomal protein, large, P2 C4orf48: chromosome 4 open reading frame 48 ZNF829: zinc finger protein 829 COX5B: cytochrome c oxidase subunit Vb COX6C: cytochrome c oxidase subunit VIc COMMD6: COMM domain containing 6 CCDC73: coiled-coil domain containing 73 C20orf52: chromosome 20 open reading frame.. USMG5: up-regulated during skeletal muscle ... RPS28: ribosomal protein S28 SNRPE: small nuclear ribonucleoprotein polyp... RPL37A: ribosomal protein L37a KLHDC8A: kelch domain containing 8A ATP5E: ATP synthase, H+ transporting, mitoc... CNKSR2: connector enhancer of kinase suppr... RPS12: ribosomal protein S12 NDUFB1: NADH dehydrogenase (ubiquinone) ...

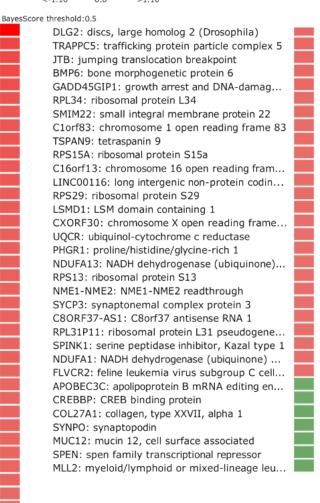


Figure S5. Heat map of the top 76 significantly perturbed genes during exposure of tomato CNTcontaining fruits to T-84 epithelial cells. Tomato fruits extracts were exposed at a concentration of 3μ g/ml. Gene expression results are based on the number of RNA-seq transcripts detected, which were normalized and expressed as fold change compared to the gene expression of cells exposed to control fruits. The fold change threshold score was selected for a 98% confidence. Darker red gradient indicates increased/higher gene expression while darker green gradient indicates suppression/lower of gene expression.

Tight junction

GENE_SYMB	GENE NAME
OL	
LIMK1	LIM domain kinase 1
ACHE	acetylcholinesterase (Yt blood group)
CLDN2	claudin 2
CTTNBP2	cortactin binding protein 2
EVPL	envoplakin
PPL	periplakin
BCAR1	similar to breast cancer anti-estrogen resistance 1; breast
	cancer anti-estrogen resistance 1
SYNPO	synaptopodin
ZYX	zyxin

Adherens junction		
	GENE_SYMB	GENE NAME
	OL	
	LIMK1	LIM domain kinase 1
	CTTNBP2	cortactin binding protein 2
	BCAR1	similar to breast cancer anti-estrogen resistance 1; breast
		cancer anti-estrogen resistance 1
	ZYX	zyxin
Desmosome		
	None	-

Table S2. Classification of genes significantly altered by exposure of CNT to epithelial T-84 cells into three categories: tight junctions, adherens junctions, and desmosomes. The gene identification and classification were performed using DAVID software analysis (DAVID Bioinformatics Resources 6.7, NIAID/NIH).

Tight junction

GENE_SYMBOL	GENE NAME
AMOTL2	angiomotin like 2
CGN	cingulin
CGNL1	cingulin-like 1
CLDN2	claudin 2
SYNPO	synaptopodin
Adherens junction	Le construction de la construction
GENE_SYMBOL	GENE NAME
LMO7	LIM domain 7
LIMK1	LIM domain kinase 1
ACTN1	actinin, alpha 1
CDH3	cadherin 3, type 1, P-cadherin (placental)
CAV1	caveolin 1, caveolae protein, 22kDa
DSP	desmoplakin
ITGA5	integrin, alpha 5 (fibronectin receptor, alpha polypeptide)
JUP	junction plakoglobin
MYH9	myosin, heavy chain 9, non-muscle
PXN	paxillin
PVRL1	poliovirus receptor-related 1 (herpes virus entry mediator C)
PVRL2	poliovirus receptor-related 2 (herpes virus entry mediator B)
PVRL4	poliovirus receptor-related 4

SCRIB	scribbled homolog (Drosophila)	
BCAR1	similar to breast cancer anti-estrogen resistance 1; breast cancer anti-estrogen	
	resistance 1	
SPTAN1	spectrin, alpha, non-erythrocytic 1 (alpha-fodrin)	
TLN1	talin 1	
TLN2	talin 2	
TNS4	tensin 4	
TRIM25	tripartite motif-containing 25	
ZYX	zyxin	
Desmosomes		
GENE_SYMBOL	GENE NAME	
DSP	desmoplakin	
EVPL	envoplakin	
JUP	junction plakoglobin	
PPL	periplakin	

Table S3. Classification of genes significantly altered by exposure of control fruits to epithelial T-84 cells into three categories: Cell adhesion, tight junction, and adherens junction. The gene identification and classification were performed using DAVID software analysis (DAVID Bioinformatics Resources 6.7, NIAID/NIH.

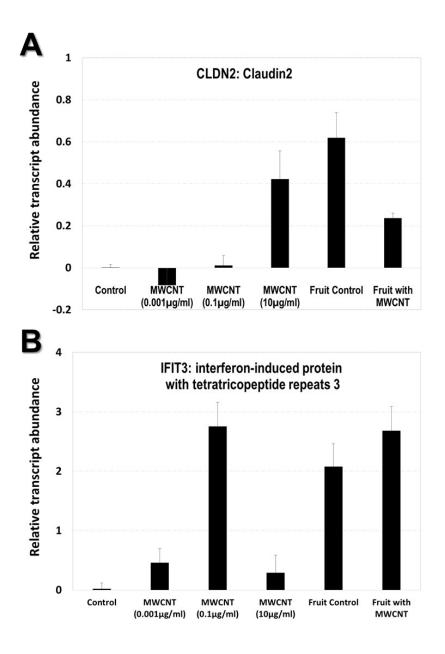


Figure S6. Real-time PCR of selected genes to confirm Next Generation Sequencing results. Genes selected included (A) Claudin2 and (B) interferon-induced protein with tetratricopeptide repeats 3. The expression of transcripts was shown as transcript abundance levels in comparison to the untreated control. Data are presented as the mean of three technical replicates, and two biological replicates (n=6). Error bars represent standard error values.

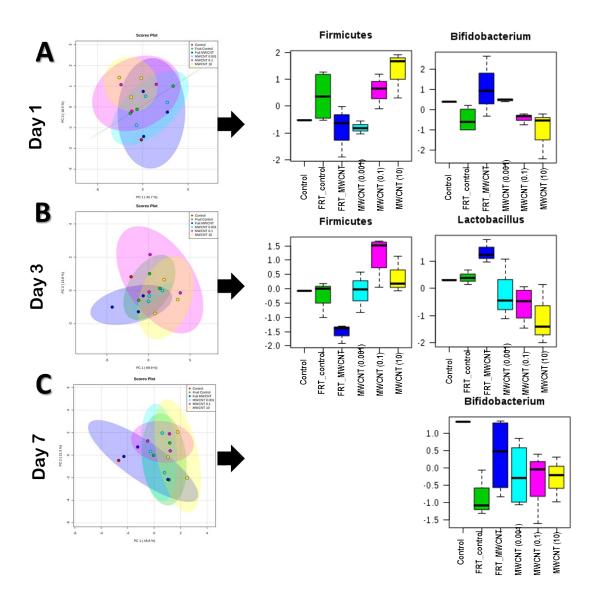


Figure S7. Principle Component Analysis of the different treatments tested on fecal slurry. One color represents each treatment, and each point represents one sample. Each cluster represents one treatment defined by a 95% limit. The analysis was performed for data obtained at day 1 (A), day 3(B) and day 7 (C). During each day, the variables contributing to the most variability in the data are presented.

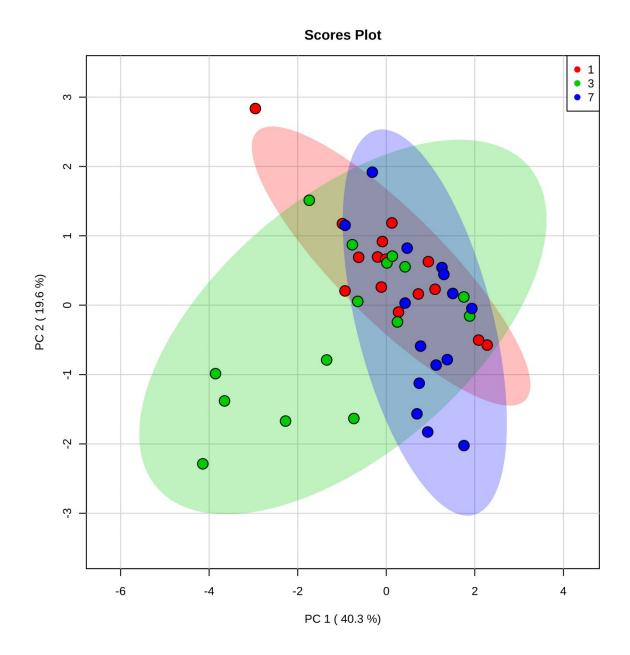


Figure S8. Principle Component Analysis of the different days of sampling. Each day (1, 3, and 7) is represented by one color, and each point represents one treatment. Each cluster represents one day defined by a 95% limit.

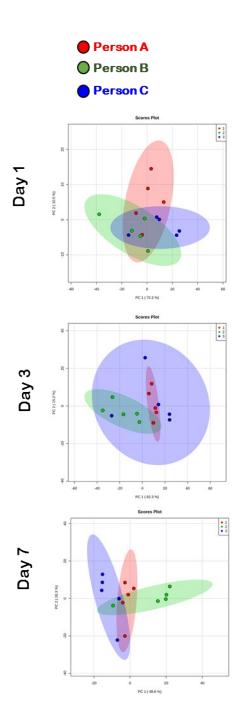


Figure S9. Principle Component Analysis of the individual A, B, and C specimens used during our exposure studies. Each sample is represented by one color, and each point represents one treatment. Each cluster represents one individual sample defined by a 95% limit. The analysis was performed for data obtained at day 1 (A), day 3 (B) and day 7 (C).