Molecular Responses of The Eukaryotic Cell Line INT407 on the Internalised *C. jejuni* - the other Side of the Coin

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Article

Molecular Responses of The Eukaryotic Cell Line INT407 on the Internalised *C. jejuni* - the other Side of the Coin

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Abstract: *Campylobacter jejuni* is a zoonotic bacterium, causing intestinal infection in humans. One characteristic feature of the pathogenicity process is the ability of this bacterium invading the epithelial cells. Several factors have been identified to contribute to this process and some recent analyses revealed changes in the bacterium cell on molecular level. However, our knowledge is still limited about responses of the host that accompany the bacterial internalisation and survival process. This study focused on molecular events on transcriptomic level detected between the 1st and the 3rd hour of internalisation in INT407 epithelial cells. From the 41.769 human genes tested, altogether 19,060 genes and 22,734 pseudogenes and introns were shown by whole transcriptome analysis to be affected in different extent. Most affected functions regulate transcription (28%), signal transduction (21%), apoptosis (15%), immune responses (9%), transmembrane transport (6%), cell-cell signaling (3%), cell-cell adhesions (3%) and carbohydrate metabolism (3%) and to a lesser extent other functions. Our results provide insight into a scenario where, the invaded cell focuses on survival, but, at the same time, flashing the possibility of pushing the process toward the death of the invaded and metabolically disrupted cell.

Keywords: *Campylobacter jejuni*; INT407 cell line infection; WTA

1. Introduction

Campylobacter jejuni is one of the most important bacterial agents causing gastrointestinal infections with varying symptoms ranging from mild to bloody diarrhea. The infection is common also in developed and under developed countries. Outbreaks were reported in Eurasia, America, Africa and even in Oceania, which means that campylobacteriosis is a global problem. Epidemics caused by *C. jejuni* are not negligible, and these are registered more frequently in developing countries [1-5].

The typical source of infection is chicken meat, but besides, infection can also be transmitted to humans through the consumption of contaminated water, -milk, and -meats, through animal contact and in general by inadequate hygienic-sanitary conditions [6, 7]. The prevalence of *C. jejuni* in India and Africa correlates with contaminated water that they drink and give to their animals [8, 9].

Living near to and together with animals is also a risk factor [10, 11], similarly to malnutrition, especially in case of children [12, 13].

Although there is a big difference among the isolates in their pathogenic potential, virulence and genome organisation, the general pathogenicity process of *C. jejuni* can be described with the following stages. *C. jejuni* enters the host intestine through the gastric acid barrier of the stomach, and colonises the mucosa covering the distal ileum and colon. During the passage into the small intestine and the migration of the bacteria towards the mucus-filled crypts, *C. jejuni* reacts, presumably as an adaptive response, to the microenvironment of the current intestinal section, where it synthesizes a new set of proteins facilitating their subsequent interaction with the host's target cells. It can penetrate

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enterocytes by paracellular or transcellular route [14, 15]. The flagellae and the screw-shape of the bacterial cells play an important role in reaching the epithelial cells through the mucus layer [16]. A group of adhesion proteins support the binding of the bacterial cell to extracellular matrix proteins (ECMPs), including fibronectin and laminin,. This process is facilitated by several factors such as CadF, Peb1, Peb2, Peb3, Peb4, CapA, CjaA, FlpA, FbpA, JlpA, DocA [17].

As the first step of the invasion the pathogen interacts with the host through biochemical signals, such as Campylobacter invasion antigens (Cia) [18, 19]. As a result, a signaling cascade triggers the rearrangement of the host's cytoskeleton, leading to the internalisation of the bacterium in a vacuole [20]. At this point a two-sided game begins in which the *C. jejuni* cell, in order to assure its survival, tries to maintain the vacuolised form by avoiding its fusion with lysosomes. On the other side, the eukaryotic cell attempts to eliminate the invader. Some recent studies have outlined molecular changes, such as the expression of capsule, lipooligosaccharide, and different membrane transport systems, and also the activation of stress related genes accompanying the invasion process and assuring the survival of the bacterial cell [17, 21, 22]. Other studies primarly focused on immunologic aspects such as the appearance of interleukines in the supernatant of invaded cells, showing increased expression of IL-8 due to infection [23] in a strain dependent manner, as well as minor changes in the expression of IL-1 and IL-4 in a time dependent manner with a slight increase at the 1st and 4th hour and a subsequent decrease by the 24th hour following infection [24]. Other studies demonstrated the massive expression of TNF-alpha suggesting that the immun response was shifted towards Th-1 type [25].

Transcriptomic methods have also been used to estimate activation of different interleukines, such as IL-4, IL-8, IL-10, also in the case of Guillan-Barre syndrome (GBS), one sequelae of *C. jejuni* infection [26-29]. However, during the invasion process, genes other than those with immunological function were not typically examined, so not much is known about the other side of the infectious process, which is how internalisation of *C. jejuni* affects the eukaryotic transcriptome of the invaded cell. This is an important issue, since the outcome of bacterial infections depends not only on the infectious agent, but also on the host itself, influencing in several cases the development of certain late onset complications following *C. jejuni* infection [30-32].

Aim of this study was to get a view about those molecular changes that occur between the 1st and 3rd hour following internalisation and to determine whether these changes help maintain or eliminate the internalised state of the *C. jejuni* cells. For this purpose a Whole Transcriptome Analysis (WTA) was performed by using the INT407 cell line and the recently isolated and partially characterised highly invasive *C. jejuni* strain CjTD-119 [17].

2. Materials and Methods

2.1. Bacterial Strains and Growth Conditions

For this study the *Campylobacter jejuni* CjTD-199 strain was used. This strain was partially characterised in a recent study [17], is a representative of a 190 piece strain collection and among the seven strains isolated from a patient with bloody diarrhoea showing strong adhesion and invasion potentials. The strain was routinely grown on Charcoal Cefoperazone Deoxycholate Agar (CCDA) at 42°C under microaerophilic conditions.

2.2. Preparing the INT 407 Cell Line for the Invasion Assay

The analyses were performed on semi-confluent monolayer of INT407 human embryonic intestine cell line grown on 24-well culture plates. For monolayers, 3x10^5 cells were cultured in RPMI 1640 medium (BioWhittaker, Lonza, Switzerland) supplemented with 10% heat-inactivated (30 min for 56 °C) calf bovine serum (Sigma-Aldrich, USA), 10,000 U/ml of penicillin, 10 µg/ml of streptomycin and 0.5 mg/ml of neomycin, and incubated overnight at 37 °C in a humidified, 5% CO_2 incubator.

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2.3. Infection of INT 407 with the C. jejuni Strain CjTD-199

Plate culture of strain CjTD-199 was suspended in RPMI 1640 and the optical density was set to OD_{600}=0.1. One ml from the suspension was added to wells containing the semi-confluent layers of INT407 cell line. Altogether 3 plates (72 wells) were used for the experiments. After addition, the bacterial cells (MOI 2), were centrifuged onto the monolayer, and the plates were incubated at 37 °C in a humidified incubator with 5% CO_2 for one and three hours. At these timepoints total RNA was isolated from the wells.

2.4. Isolation of RNA from the INT 407

RNA isolations from the INT407 cells were performed as described earlier [17] with some modifications. Briefly, before collecting the cells wells were washed once with PBS. After removing not invading *C. jejuni* cells, 1 ml RNAprotect Bacteria Reagent (Qiagen, Hamburg, Germany) was applied to stabilize the RNA in each well. Following 5 minutes of incubation at room temperature, cells were trypsinized (Life Technologies, Carlsbad, California, USA) and detached cells were collected with centrifugation (2000 × *g* for 5 min). Collected cells were homogenized in RNAzol (Molecular Research Center, Cinncinatti, Ohio, USA) in a 1.5 ml microcentrifuge tube. The tubes were dropped three times into liquid nitrogen (-196 °C) for more effective extraction of the RNA. The total RNA concentration and purity was measured by an ND–1000 Spectrophotometer (Nanodrop, Thermo Scientific, Carlsbad, California, USA).

These isolation steps were performed on the relevant plates at the 1st and 3rd hour after infection.

2.5. Whole Transcriptome Analysis

WTA was performed as described earlier[17]. Qualitative and quantitative measurements of the isolated RNA were carried out with Bioanalyzer (Agilent Technologies, Santa Clara, California, USA) and Qubit (Life Technologies). High quality RNA samples were pooled for analysis. We used the SOLiD total RNA-Seq Kit (Life Technologies, USA) and RiboZero Prokaryotic rRNA Removal Kit (Epicentre, USA). Remaining RNAs were fragmented by using RNase III, and the 50-200 nucleotide long fragments were ligated to adaptors. Reverse transcription of these constructs was performed with ArrayScript RT kit (Thermo Fisher Scientific, USA). The Qiagen MinElute PCR Purification Kit (Qiagen, Germany) was used to purify the cDNA library and the SOLiD Library TaqMan Quantitation Kit (Life Technologies, USA) was used to determine library concentration. Emulsion PCR (ePCR) was used to amplify quality controlled libraries on SOLiD P1 DNA Beads, followed by enrichment for template-positive beads by hybridization with magnetic enrichment beads. Template-enriched beads were extended at the 3′ end in the presence of terminal transferase and a 3′ bead linker. Beads with the clonally amplified DNA were deposited onto sequencing slides and sequenced on a SOLiD5500XL Instrument (Thermo Fisher, Carlsbad, California, USA) using the 50-base sequencing chemistry.

2.6. Bioinformatics - Heatmap

The CLC Genomics Workbench (Qiagen) was used for data analyses. Low quality and short sequences were removed in a trimming step, and only sequences 45–50 nucleotides long were used for analysis. Sequences were mapped onto the human eukaryotic genome sequence. Results were manually cured to remove false positive hits, which showed highly skewed mapping of reads. Only genes, detected with at least a 2.0-fold increase or decrease in transcription level after normalization were considered for further analysis. For heatmap analysis, normalized expression values of genes for the two samples were plotted on a heat map using the pheatmap v1.0.12 package in the R language (v4.2.2). Genes with FDR p-value corrected Kal's Z-test p-values <0.001 were taken into acccount. The fifty most upregulated and fifty most downregulated genes, based on fold change of normalized values, were selected for visualization.

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3. Results

3.1. Comparison of Expression Changes of Genes Associated with Immune Functions

The complete list of the 41.796 genes with their expression levels are listed in the Supplementary Table 1. Altogether 19.060 genes and 22.734 pseudogenes and introns were identified by whole transcriptome analysis by mapping them on to the human genom. From among the active genes 2.764 were upregulated (fold change >2.0), while 2.220 were downregulated (fold change >2.0) in the two hours' time range between the 1st and 3rd hour after *C. jejuni* cells became internalized into the INT407 cell. Grouping of the genes with increased or decreased expression levels, based on their formerly confirmed or only hypothesized major physiological functions are summarized in Figure 1.

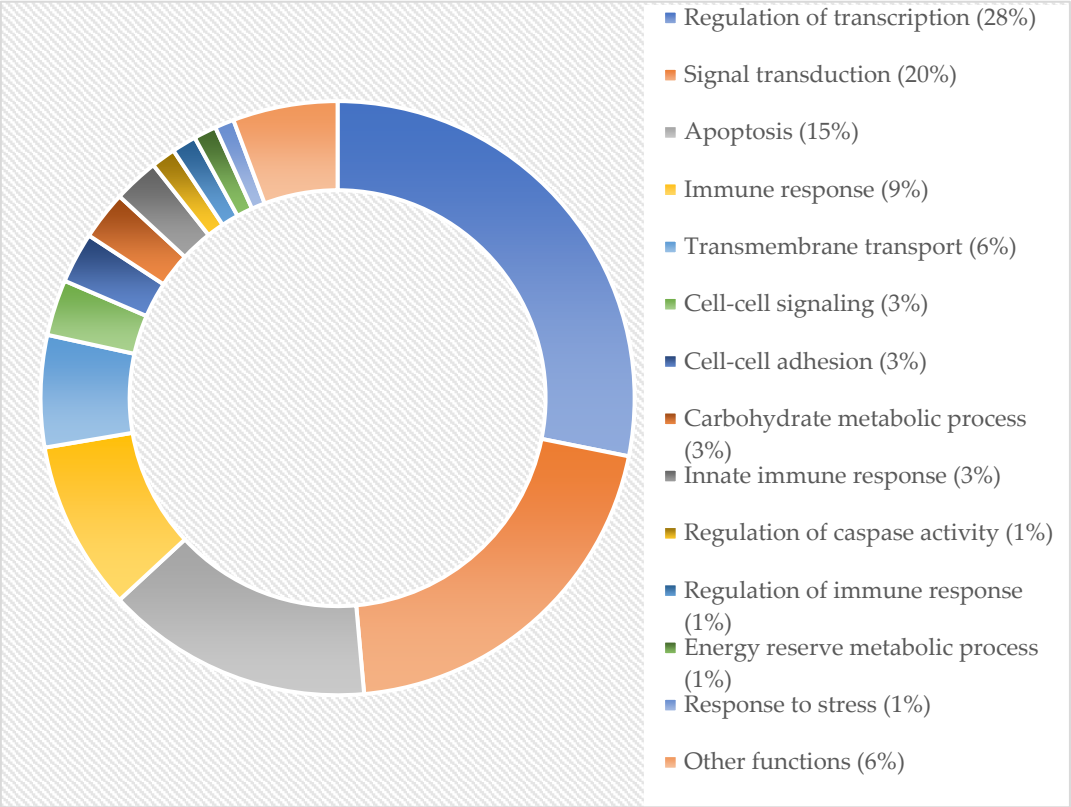

Figure 1. Genes of the *Campylobacter jejuni* strain CjTD-199 affected between the 1st and 3rd hour of the invasion process. Other functions are the following: response to toxin, response to oxidative stress, receptor-mediated endocytosis, stress-activated MAPK cascade, peptidyl-tyrosine phosphorylation, cell-cell junction organization.

Genes associated with immunological functions showing an altered expression are presented in Table 1.

Data show that the infected human cells reacted to the *C. jejuni* infection with an upregulated immune response. Not only the genes responsible for innate, but also some others taking part in adaptive immune processes were upregulated. The gene showing the highest expression change was *ulbP3* (ULBP3, Fc.: 10.10x), whose protein product ULBP3 is related to MHC class I proteins and is an important regulatory protein of the natural immune system. Another gene with strongly upregulated expression was *cr1* (CR1; Fc.: 7.581x), coding for a transmembrane glycoprotein playing part in various processes in the body, including adhesion and phagocytosis of immune cells [33] and also associated with several diseases [34]. A gene responsible for macrophage activation, *ifi44L*, showed a characteristic increase (IFI44L, Fc.: 3.685x) which was among the highest in the group of genes affecting immune processes.

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A slightly lower expression change (CD74, Fc.: 2.527x) was detected in the case of *cd74*, that performs many tasks in the eukaryotic cell. Its functions include (i) negative regulation of mature B cell apoptosis, (ii) positive regulation of neutrophil chemotaxis, (iii) positive regulation of T-helper type 2 immune response, and negative regulation of apoptosis [35]. Additionally, it is a positive regulator of B-cell proliferation, and the extracellular signal-regulated kinase 1/2 (ERK1 and ERK2) and mitogen-activated protein kinases (MAPKs), both of them taking part in a diverse array of cellular processes [36].

Expression levels of four genes *cd36*, *procr*, *cd209*, *il10ra* (CD36, Fc.: 2.73, PROC, Fc.: 2.527x; CD209, Fc.:2.52; IL10RA, Fc.:2.52), all responsible for antigen processing and presentation were also markedly increased. CD36 is an exogenous peptide and has a role in antigen presentation via MHC class I, and positive regulation of the MAPKKK cascade. The function of CD209 (CD209, Fc.: 2.53x) is: regulation of T-cell proliferation, antigen processing and presentation, and innate immune response [37]. IL10RA is the cell surface receptor for the cytokine IL-10 [38].

IL23R (IL23 receptor, Fc.: 6.31x) is the cell surface receptor for the cytokine, that regulates the activity of immune cells. When IL-23 binds to its receptor, it triggers a series of chemical signals inside the cell. These signals promote inflammation and help coordinate the immune system's response to foreign invaders such as bacteria and viruses.

Table 1. Affected genes associated with immunfunctions.

Gene Name	**Function**	**1vs3 Experiment - Fold Change (normalized values)**	**Refer.**
ULBP3	regulation of immune response, natural killer cell activation	10.1090	[39] Sun 2013
CR1	innate immune response phagocytosis	7.581748	[40] Fällman et al. 1996
IL23R	positive regulation of defense response to virus by host, inflammatory response	6.31	[41] Lupardus et Garcia 2008
EDN2	macrophage activation, signaling patway	3.68557	[42] Grimshaw et al 2002
IFI44L	defense response to virus, immune response	3.317	[43] DeDiego et al.2019
CD74	negative regulation of mature B cell apoptosis, positive regulation of neutrophil chemotaxis, positive regulation of T-helper 2 type immune response, T cell selection, negative regulation of apoptosis, positive regulation of B cell proliferation, positive regulation of ERK1 and ERK2 cascade, antigen processing and presentation	2.5272	[35] Su et al.2017 [44] Starlets et al.2006
CD36	antigen processing and presentation of exogenous, peptide antigen via MHC class I, antigen processing and presentation of peptide antigen via MHC class I	2.73785	[45] Urban et al. 2001
PROCR	antigen processing and presentation, PROCR acted as a negative regulator of Th17 pathogenicity	2.5272	[46] Kishi et al. 2016

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CD209	regulation of T cell proliferation, antigen processing and presentation, innate immune response	2.5272	[47] Preza et al. 2014
IL10RA	inhibits the synthesis of proinflammatoric cytokines	2.5272	[48] Liu et at. 1994

3.2. Affected Genes Related to Metabolic Functions

Among the metabolism-related genes, we found four whose expression was changed more than 2 fold over the study period. $ABCD_2$ (ABCD2, Fc.: 11.37x) has a key role in the fatty acid homeostasis of peroxisomes, the oxidative organelles of eukaryotic cells [49]. TRPM6 (TRPM6, Fc.: 11.37x) is a selective magnesium channel [50], while ENPP3 (ENPP3, Fc.: 8.84x) is an ectoenzyme, that hydrolyses extracellular nucleotides, such as ATP, preventing ATP induced apoptosis and deregulates cytokine production.

Several other metabolism-related genes were found to be up and downregulated (Table 2.) with changes less than 2.0 in expression level.

Table 2. Genes with metabolic functions.

Gene name	Function	Fold change	References
ABCD2	very-long-chain fatty acid metabolic process	13.89	[49] Fourcade et al.2009
TRPM6	Mg^{2+} channel, and uptake regulator	11.37	[51] van der Wijst et al.2014
ENPP3	phosphate metabolic process, nucleoside triphosphate catabolic process	8.84537	[52] Tsai et al. 2015
GFPT2	glutamine metabolic process, fructose 6-phosphate metabolic process	2.52	[53] Wang et al.2022

3.3. Comparison of Expression Changes of Genes Associated with Stress Responses

Concerning stress related genes, a drastic increase in the expression of *vnn1* (VNN1, Fc.: 379.08x) was detected. This gene's product contributes to tolerance to tissue damage and modulates the ability of the affected cell to cope with oxidative stresses. Additionally, another stress gene *lpo*, first described in connection to lipid peroxidation, and strongly related to oxidative stress conditions showed increased expression at the 3rd hour following infection (LPO, Fc.: 5.05x). Furthermore, a slight increase in the expression of 3 additional genes: *chac1*, *adcyap1r1* and *rgcc* was detected (CHAC1, Fc.: 8.845x; ADCYAP1R1, Fc.: 3.791x; RGCC, Fc.: 3.791x). CHAC1, is thought to regulate the glutathion level and also oxidative balance in the cell. Lipid peroxidation, controlled by LPO is a fundamental constituent of oxidative stress and free radical production [54], while RGCC has an effect on stress fiber formation [55]. Expression of HSPA12B (HSPA12B, Fc.: -2.374x) a heatshock protein [56], and SCAMP5 (SCAMP5, Fc.: -3.165x) an inhibitor of endocytosis were downregulated during the investigated time period [57].

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Table 3. The expression changes of genes associated with stress conditions.

Gene	Function	1vs3	Ref.
VNN1	response to oxidative stress, pantothenate metabolic process	379.08	[58] Zhang et al. 2017
CHAC1	apoptosis in response to endoplasmic reticulum stress	8.84537	[59] Mungrue et al. 2009
LPO	response to oxidative stress	5.05	[60] Kovács et al. 1996
ADCYAP1R1	multicellular organismal response to stress	3.791	[61] Ressler et al. 2011
RGCC	positive regulation of stress fiber formation, cell cycle regulation	3.791	[62] Wang et al. 2011
HSPA12B	response to stress	-2.374122	[63] Zouein et al. 2013
SCAMP5	response to endoplasmic reticulum stress	-3.165496	[57] Noh et al. 2009

3.4. Affected Genes Related to Apoptosis

At least 25 genes formerly hypothetised to have roles in the induction of apoptosis were also affected with more than 2 fold expression change. Among these, 22 were found to influence the apoptopic process positively.

Concerning the positive regulators of apoptosis, the expression of *dcc* (DCC, Fc.: 5.68x), *dlc1* (DLC1, Fc.: 5.05x), *cd27* (CD27, Fc.: 3.79x) has been documented in several different cells, while the expression of other genes, such as *fndC1* (FNDC1, Fc.: 7.85), until now has only been detected in specific cell types, such as cardiac cells. *FndC1* plays an important role in angiogenesis and is essential to hypoxia-triggered cardiomyocyte apoptosis [64]. Recent studies, however, demonstrated that aberrant expression of *fndC1* is associated with tumorigenesis, for example, in the case of gastric cancer [65].

Other affected genes are inhibitors of apoptosis. It was reported, that *serpinB9* (SERPINB9, Fc.: 11.37x) protects cells from the immune-killing effect of granzyme B (GRB) released by lymphocytes [66].

Table 4. Genes that play a role in apoptosis.

Genes.	**1vs3**	**Funciton**	**References**
SERPINB9	11.3726	negative regulation of apoptosis by inhibiting granzyme B	[67] Bird et al. 2014 [68] Kaiserman et al. 2010
ACVR1C	10.1089	regulation of apoptosis	[69] Asnaghi et al. 2019
CHAC1	8.8454	apoptosis in response to endoplasmic reticulum stress	[70] Zhou et al. 2023
FNDC1	7.8517	positive regulation of cardiac muscle cell apoptosis	[71] Das et al. 2017 [72] Yunwen et al. 2021
G0S2	6.9499	positive regulation of apoptosis	[73] Heckmann et al. 2013

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NFATC4	6.31812	positive regulation of apoptosis	[74] Mognol et al. 2016
HIC1	5.8969	signal transduction resulting in induction of apoptosis	[75] Wang et al. 2017
DCC	5.6863	regulation of apoptosis	[76] Mehlen et al. 1998
DLC1	5.0545	induction of apoptosis	[77] Zhang et Li 2020 [78] Ullmannova et al. 2007
CD27	3.79087	induction of apoptosis	[79] Prasad et al. 1997
CASP3	2.7851	nuclear fragmentation during apoptosis	[80] Porter et Jänicke 1999

3.5. Genes Involved in the Potential Development of Chronic Conditions

Expression change of several genes previously hypothesised or verified to have potential roles in the evolvement of post-infectious pathological conditions was revealed in our study. Four genes, formerly thought to influence the severity of Guillan-Barre syndrome (GBS) [81-83] were affected differently but in a moderate way (PTGS2, Fc.: 1.188x; ANXA3, Fc.: 1.31x; CREB1, Fc.: 1.73x) by the 3rd hour of infection (Table 5). Characteristic upregulation of the genes *relb*, *birc3* and *nfkbia* (RELB, Fc.: 1.625x; BIRC3, Fc.:1.958x; NFKBIA, Fc.:-2.55x), associated with the progression of inflammatory reactions, was detected. However, a higher expression rate was seen in the case of *ace* (ACE, Fc.: 3.79x), a gene hypothesised to induce autoreactive TH1 and TH17 cells and suppress regulatory T cells, and thus beeing involved in autoimmune responses of the body[84].

Another characteristic group of genes whose expression was found to be altered in the investigated time range and which have previously been shown to be associated with pathological condition, are genes linked to tumorgenesis. The *muc4* and *muc6* (MUC4, Fc.: 3.15x; MUC6, Fc.: 3.36x) showed clear increases in their expressions, with values of 3.15x and 3.36x respectively. A more drastic expression was detected in the case of three genes *serpinB9*, *fndC1*, *tacrD2* (SERPINB9, Fc.: 11.37x; FNDC1, Fc.: 7.58x; TACRD2, Fc.: 8.84x). SerpinB9 has been demonstrated to be significantly associated with the development of precancerous lesions [66]. In contrast, *fndC1* promotes the invasiveness of gastric cancers and correlates with the appearance of peritoneal metastasis [65].

Table 5. Genes involved in the potential development of chronic conditions.

	Gene name	**1vs3**	**referenc.**
Guillan- Barré s. disease severity (GBS)	PTGS2	1.188331	[83] Chang et al.(2012)
	ANXA3	1.315609	[82] Hughes et al. (1978)
	CREB1	1.732517	[82] Hughes et al. (1978)
Inflammatory	RELB	1.624660	[85] Breuer et al. 2013
	BIRC3	1.958618	[85] Breuer et al. 2013

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	NFKBIA	-2.553647	[85] Breuer et al. 2013
Autoimmune inflammation	ACE	3.79087	[86] Connell et al. 2012
General cancer markers	TLR3	3.79087	[87] Wang et al. 2015
	CD36	2.73785	[88] Wang et Li 2019
Tumorgenesis	SERPINB9	11.37262	Wang et al. 2021
	FNDC1	7.581749	Jiang et al. 2020
	TACR2	8.845373	[89] Yu et al. 2012 [90] Jianfeng et al. 2021
Gastric cancer	GALNT5	8.84537	[65, 91] Jiang et al.2020 Guo et al.2018
	MUC6	3.36	[92] Marín et al. 2012
Pancreatic cancer	KRAS	2.011985	[93] Chang et al. 2020
	SMAD4	1.34157	[94] Xia et al. 2015
	BRCA2	1.231316	[95] Naderi et Couch. 2002
	NBL1	5.054499	[96] Olakowski et al. 2009
	MUC4	3.15	[97] Singh et al. 2007
Oxidative stress in the intestine	VNN1	379.087	[98] Pinho et al. 2022 [99] Kang et al. 2016

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3.6. Markedly Affected Genes with Unknown Functions

Table 6.

Gene name	Fold change
ST20-MTHFS	18.95437
PRR4 (NW_003571047 44554..48182)	11.3726
C12orf55	10.109
NDUFA3(NW_003571054 77272..81394)	10.109
COL11A2 (NT_167245 4411775..4441552)	7.581748
ADCK5 (NT_037704 165142..185869)	3.79087

3.7. Heat Map Analysis

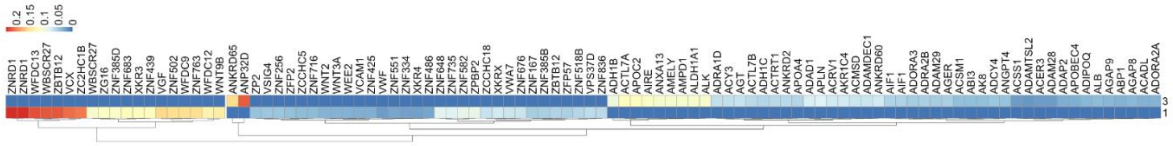

Figure 1. Visualisation of the relative expression ratios of the fifty most upregulated and fifty most downregulated genes based on the normalized fold change expression values.

4. Discussion

Up-, and downregulation of the 2.764 and 2.200 genes, respectively, from the 41.769 ORFs reflects intensive transcriptomic changes in the INT407 intestinal human cell line upon the invasion of *C. jejuni*. Our preliminary findings have revealed that by the first hour after infection, 85% of the *C. jejuni* cells became stably internalised. From that point the possible routes for elimination of the invader can be outlined.

Activation of immune functions was represented by the increased expression of several relevant gene products. Overexpression of ULBP3 (ULBP3, Fc.: 10.109x) (Table 1), encoding for an MHC I related cell surface protein, clearly indicates that the invaded cell prepares itself for antigene presentation, which is a crucial step in the immune recognition of cells infected with viruses or invasive bacteria [40]. Due to antigen presentation, cytotoxic T cells are able to recognise infected eukaryotic cells [100]. However, a proper processing of antigens is a prerequisite for this, in which the recently discussed [45-47] role of *cd36 (*CD36, Fc.:2,74x), *procr* (PROCR, Fc.:2.53x), *cd209* (CD209, Fc.:2.523x) and *il10ra* (IL10RA, Fc.: 2.53x) is also supported by our results. Antigene presentation on the surface of the affected eukaryotic cell can not be effective without attracting immune cells, which makes the increased expression levels of *cr1* (CR1, Fc.:7,59x) and *ifi44L* (IFI44L, Fc.: 3,317x) [40, 43] not surprising, since these proteins make the infected cell more accessible to macrophages [33]. Cytokines and interleukins play an important role in the attraction of immune cells and coordination of immunological processes for which the expression of the proper receptors is inevitable. Increased expression rates of the IL10 (IL10RA, Fc.:2.53x) and IL23 (IL23R, Fc.:6.31x) (Table 1) receptors strongly suggests that these two interleukins contribute to the immune reaction evoked by the vacuolised pathogen. IL10RA activates the tyrosine phosphorylation of JAK1 and TYK2 kinases, two enzymes contributing to the alteration of the IFN-alpha/beta and gamma signaling pathways, thereby affecting the production of certain groups of cytokines [101]. The mild expression change of IL4 (IL4I1, Fc.: 1.25x) was in accordance with the findings of other authors [25], while the decreased expression of IL8 (IL8, Fc.:-2.74x) by the 3rd hour of infection is seemingly in contrast with an earlier finding [23]. A plausible explanation would be that our data were generated from the 3rd hour, while in the study of

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Hickey , samples were taken 24 hours following infection. Another possible explanation may be the strain dependent manner of the IL8 expression rate [23]. In a recent study the steep increase of the proinflammatoric citokines IL6, IL8 and IFN-γ and that of a regulator citokine IL10 was detected from the 5th hour following infection [102]. Our results are in partial accordance with these findings, showing moderate increases in the case of IL6 (IL6R, Fc.:1.7x), IL10 (IL10RA, Fc.:2.52x) and IFN-γ (IFNGR2, Fc.:1.4x), while the level of IL8 transcript dropped (IL8, Fc.:-2.74x) in the investigated time range (STable 1.)

Based on our data, campylobacter infection outlines two possibilities for the invaded eukaryotic cell. One is survival by activating the immune system, while the other option is to sacrifice the eukaryotic cell by apoptosis in order to eliminate the pathogen from the body [103]. In certain situations, for an infected cell it is much more rewarding to drive itself toward apoptosis, a notion supported by the upregulation of 12 genes listed in Table 4. In contrast, expression of *serpinB9* and *cd74* acts against apoptosis (Table 1), of which the latter one, as a positive regulator of the type-2 immune response [44], directs the process toward activation of adaptive immune processes.

Be it the activation of the immune system or apoptosis, both potential routes drastically reprogram metabolism and with that, require either the activation or the depression of transcription factors (STable 1). The coordinated increased expression of *znf491* (ZNF491, Fc.:2.52x), *znf560* (ZNF560, Fc.:2.52x), *znf516* (ZNF516, Fc.:2.52x)[104], *esrrg* (ESRRG, Fc.:2.52x) [105] and the enhanced expression of translation and post-translation factors, such as GALNT5 (GALNT5 Fc.:8.84x), MUC3A (MUC3A, Fc.:6.31x) MUC6 (MUC6 Fc.:3.36x) , MUC4 (MUC4 Fc.:3.15x) [106-108], PIWIL3 (PIWIL3 Fc.:3.79x), PIWIL4 (PIWIL4, Fc:3.79x, F9 (F9, Fc.:2,52x) and GFPT2 (GFPT2 Fc.:2.52x) support the existence of this highly accelerated metabolism. Downregulation of NDUFA13 (-4.11x), a negative regulator of translation [109], suggests that also the activity of genes or group of genes controlled by this regulator is crucial in the battle between the invader and the host. During this encounter, maintenance of the intracellular homeostasis is crucial that is represented by the increased expression of TRPM6 contributing to Mg homeostasis (Table 2) a key element of several enzymatic functions.

The markedly activated levels of CHCA1 (CHCA1, Fc.:8.84x)[59] and LPO (LPO, Fc.:5.05) [60] showed that the eukaryotic cell containing the vacuolised *C. jejuni* 1-3 hours after infection underwent a marked stress situation. Decreased expression of CHRNE, a regulator of membrane potential (CHRNE, Fc.:-4.74x), assumes the development of an osmotic shock. On the other hand, the drastically decreased expression of GAPDHS (GAPDHS, Fc.:-4.43x), an enzyme taking part in glycolysis [111], clearly indicates a partial slowdown of some parts of the metabolic machinery of the eukaryotic cell.

Stress situations are characterised by the fact that the cell pauses its non-essential activities and focuses on saving energy. One feature of this may be the activation of the cell cycle arrest gene, *sesn2* (SESN2, Fc.:3.23x) [112], thus stopping proliferation, an energy consuming process. Another example for energy saving is the decreased expressions of PARVG (PARVG, Fc.: -3.95x) [113] taking part in matrix protein synthesis and matrix protein processes. The reduced expression of matrix proteins could either facilitate the killing of the infected cell by making itself more accessible and at the same time more vulnerable to the damaging enzymes of macrophages. This hypothesis is supported by the powerful increase of EDN2 (EDN2, Fc.:3,68x) [42] a macrophage chemoattractant and the increased expression of the formerly mentioned ULBP3, responsible for natural killer cell activation.

The decreased expression of FAM132A, a negative regulator of inflammation [114], suggests an induced inflammation in the invaded INT407 cell. Activation of *relb, birC3, nfkbia* [115] further supports this observation. It is important to note, that as a consequence of bacterial infections inflammatory diseases and other pathological conditions, such as tumors can develop. In this context, *C. jejuni* infection has been implicated in the development of Guillan-Barre and Miller-Fisher syndromes (GBS and MFS). Reason of the more or less unaffected expression levels of genes coding for, *ptgs2* (PTGS2, Fc.:1.2x), *anxa3* (ANXA3, Fc.:1.32x) and *cerb1* (CERB1, 1.73x) (Table 5.), four gene products considered to be associated with GBS, may be that the effects of the affected genes manifest themselves only after a long period of time or, as it was recently suggested, they are associated only with certain *C. jejuni* serotypes [81, 82, 116].

The slight increase in the expression levels of genes associated with tumor genesis supports recent assumptions and findings that certain bacterial infections increase the risk of developing malignant tumors in the colon [117], the biliary tract [118], and the esophagus [119]. Although tumor genesis is a complex process about which our knowledge is still limited, the high expression levels of *serpinB9* (SERPINB9, Fc.: 11.37x) and *tacr2* (TACR2, Fc.: 8.84x) [66, 90], two proteins associated with tumor formation, supports the potential role of *C. jejuni* in the development of these pathological conditions.

5. Conclusions

Results of this study show that the expression of a wide repertoire of genes in the INT407 epithelial cell line changes during invasion of *C. jejuni*. Further studies can clarify the roles of concrete genes during the invasion process, and could help to reveal those till unknown molecular processes by that certain cell types, like macrophages, are able to defeat this zoonotic bacterium, in contrast epithelial cells where eliminiation of *C. jejuni* is inhibited.

Supplementary Materials: The following supporting information can be downloaded at: www.mdpi.com/xxx/s1, Figure S1: title; Table S1: title; Video S1: title.

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