THE INTERPLAY OF TLR9-MEDIATED AUTOPHAGY RESPONSE AND GROWTH FACTOR SIGNALING INHIBITION IN COLON ADENOCARCINOMA CELLS: CELL-FREE DNA EXPERIMENTS

PhD thesis (short version)

Bettina Aranka Bohusné Barta

Semmelweis University Doctoral School

Károly Rácz Conservative Medicine Division

Supervisor: Ferenc Sipos MD, Ph.D

Consultant: Györgyi Műzes MD, C.Sc, med. habil.

Official reviewers: Pál Miheller MD, Ph.D, med. habil.
Gábor Rubovszky MD, Ph.D

Head of the Comprehensive Exam Committee:

Zoltán Prohászka MD, D.Sc

Members of the Comprehensive Exam Committee:
Miklós Máté MD, Ph.D
Gábor Firneisz MD, Ph.D

Budapest
2024

1. INTRODUCTION

Inflammation is an essential element in fighting infections, in the process of wound healing, and in maintaining tissue homeostasis. Since 2009, it has been known that inflammation, the "seventh hallmark" of cancer, affects all phases of tumorigenesis (e.g, initiation, promotion, invasion, metastasis, and immune escape). Inflammation can cause DNA damage or genetic instability as a cellular stressor. Chronic inflammation can also induce genetic mutations and epigenetic changes that promote the malignant transformation of cells. Understanding the relationship between inflammation and tumorigenesis and the role of cell-free DNA (cfDNA) released from cells in influencing this relationship could help to explain the pathogenesis of tumorous diseases as well as provide a basis for treatment and diagnostics. The aim of my PhD work was to provide an insight into the complex immunobiological effects of cfDNA in colon adenocarcinoma cells.

1.1 The origin and characteristics of cfDNA

It has been known for decades that cfDNA sequences are present in the circulation. Their origin can be from tumor cells and non-tumor cells. cfDNA is partly released by cell death and clearance mechanisms (e.g., apoptosis or autophagy) and through active secretion (e.g., DNA-protein complexes, extracellular traps, exosomes). cfDNA can increase in quantity and change in quality in physiological states, such as pregnancy or sports, and in acute and chronic inflammatory conditions, like sepsis, autoimmune diseases or tumors. The methylation pattern of cfDNA is indicative of its origin. Additionally, cfDNA has immunomodulatory effects, influencing the inflammatory process and tumorigenesis.

1.2 Recognition and immunomodulatory role of cfDNA

cfDNA acts as a DAMP and can be sensed by three receptors (i.e., cGAS, AIM2, and TLR9). Class III PI3K promotes the internalization of cfDNA and CpG-ODNs into TLR9-containing endosomes. Due to the cfDNA-TLR9 interaction, MyD88 binds to the TIR domain of TLR9, causing IRAK-TRAF6 complex activation. Finally, activation of the MAPK and IKK inhibitor complex results in increased *NF-ΚB* and *AP1* expression and induction of autophagy, among others.

1.2.1 Features of TLRs and TLR9 signaling

TLRs are pattern recognition receptors of the innate immune system. The intracellular TLRs (TLR3, -7, -8, -9) specifically sense nucleic acids of different origins (ssRNA, dsRNA, and methylated CpG-DNA). TLR9-binding CpG-DNA has immunostimulatory effects. The TLR9-dependent stimulation of native immune responses by CpG DNA has been investigated in certain infections and tumorous diseases.

1.3 Characteristics and process of autophagy

Autophagy is an evolutionarily conserved, multistep process of cellular self-digestion involving the sequestration and lysosomal degradation of accumulated, damaged, or senescent proteins and intracellular organelles in autophagosomes. It affects many biological functions, including cell development, differentiation, survival, and aging. In addition to maintaining eukaryotic cell homeostasis, autophagy is a regulatory process that profoundly influences infectious and non-infectious inflammatory processes and native and adaptive immune responses. Disruption of autophagy function is part of the pathomechanism of many pathologies, including tumors.

1.3.1 Autophagy and TLR9 signaling in cancer

The TLR9 signaling pathway and autophagy interact in the cellular responses to PAMPs and DAMPs. Their regulatory signaling link is crucial for preventing inadequate or excessive immune responses. Activation of TLR9 enhances the function of autophagy, leading to increased cell survival. Autophagy also contributes to cell death by facilitating the presentation of endogenous cytosolic proteins through MHC II, such as when bacterial CpG patterns increase the presentation of tumor antigens, potentially boosting the immune response against tumors. The exact function of the TLR9-autophagy connection in carcinogenesis is not completely understood, although it is believed to be a crucial one.

1.4 cfDNA in tumors

CfDNA sensing can exert both pro- and anti-tumor effects. It promotes tumor cell division, growth, migration, invasion, adhesion, tumor "immune escape" mechanisms, and the development of a pro-tumor peritumoral environment. However, it can stimulate antitumor

immunity, dendritic cell maturation, antigen presentation, antitumor inflammatory mechanisms, cytotoxic T cell activity, and apoptosis by inhibiting cell growth, migration, and survival.

1.5 HGFR: functions, relationship with autophagy and cancer

A variety of cell types, including tumor cells, express HGFR, a transmembrane RTK. Its pleiotropic ligand is HGF. The *HGF/HGFR* system promotes cell survival, tissue repair, migration, and adhesion and also has anti-inflammatory effects. HGFR can be activated via the canonical (dimerization with another receptor) and conventional pathways (HGF-HGFR binding and homodimerization). HGFR activation in CRC influences tumor cell survival, proliferation, motility, migration, invasion, and EMT. Inhibition of HGFR in cancer cells can activate and inhibit tumor-promoting autophagy, thus making it a promising anticancer treatment alternative.

1.6 IGF1R: functions, relationship with autophagy and cancer

IGF1R is a transmembrane RTK ligated with insulin and IGF1/2. IGF1R activation regulates apoptosis and cell growth under physiological conditions, has a protumor effect in tumors, and plays a role in the maintenance of the transformed phenotype, tumor cell growth, migration, and EMT. When IGF1R is inhibited, concomitantly induced cell protective autophagy stimulates cell proliferation and inhibits apoptosis. Combining IGF1R inhibition with autophagy inhibitors blocks autophagy, leading to decreased cancer cell proliferation and increased apoptosis. Targeted inhibition of *IGF1R* can stimulate protective autophagy while negatively affecting autophagosome formation. This is partly the basis for the fact that many cancers respond well to IGF1R inhibitor treatment.

2. OBJECTIVES

1. To select a human colorectal cancer adenocarcinoma cell line that is suitable for the combined application of cfDNA-induced TLR9-mediated autophagy and HGFR/IGF1R inhibition.

2. To investigate the complex biological effects of TLR9-mediated autophagy and HGFR inhibition induced by cfDNA:
How do cfDNA treatments with different properties (i.e., genomic, fragmented, or hypermethylated) affect the metabolic activity, proliferation, autophagy response, and stem cell phenotype of the selected colorectal cancer adenocarcinoma cell line?

3. To investigate the complex biological effects of TLR9-mediated autophagy and IGF1R inhibition induced by cfDNA:
How does genomic cfDNA treatment affect the metabolic activity, proliferation, autophagy response, and stem cell phenotype of the selected colon cancer adenocarcinoma cell line?

3. METHODS

3.1 Selection and maintenance of HT29 cell culture; self-DNA isolation

Cell line selection based on the following criteria: A., the presence of a basal, inducible TLR9 expression; B., the *MyD88*-dependent and -independent *TLR9* signaling pathways are intact; C., the presence of a moderate/high basal HGFR and IGF1R expression; D., the presence of TLR9 and autophagy-mediated HGFR cross-activation; E., the presence of an elevated IGF2 expression. F., the selected cell line adequately represents sporadic colorectal cancers; G., the used inhibitors do not exhibit significant cell proliferation inhibition. HT29 colon adenocarcinoma cells fulfill these criteria.

Genomic DNA (gDNA) was isolated from $5x10^7$ steady-state, proliferating HT29 cells. DNA concentration was determined by Nanodrop. Gel electrophoresis revealed that the gDNA fragment length was approx. 10,000 base pairs.

3.2 Fragmentation and hypermethylation of gDNA

gDNA was divided into three equal shares; the first one was neither fragmented nor hypermethylated (gDNA). The second one was fragmented (fDNA) by ultrasonic fragmentation. The third share was hypermethylated (mDNA) using M.SssI CpG methyltransferase. The length of the f- and mDNAs was determined by agarose gel electrophoresis.

3.3 HT29 cell treatments

$5x10^5$ HT29 cells were seeded in a 12-well plate with RPMI 1640 supplemented with amphotericin B, gentamycin and FBS. After 24 hours, the medium was changed, and separate aliquots of 15μg of modified gDNAs were added. The incubation time was 72 hours.

3.4 Inhibition of receptor signaling and autophagy

For inhibition of TLR9-, HGFR-, IGF1R-signaling or autophagy, HT29 cells were pretreated with different combinations of the TRL9 inhibitor ODN2088 (O; 5μM), the HGFR inhibitor 4,4'diisothiocyanatostilbene-2,2'-disulfonic acid (DISU, D; 4μM), the IGF1R inhibitor picropodophyllin (P; 0.05μM) or the autophagy inhibitor chloroquine (C; 10 μM) for 1 hour before DNA treatments.

3.5 Cell viability and proliferation measurements

The use of the Alamar Blue assay served a dual purpose: partly to examine cell viability (metabolic activity) and partly to study cell proliferation.

Cell viability after the 72-hour long treatments were measured by a 4-hour incubation period using Alamar Blue. The fluorescence was measured at 570–590nm and the results were analyzed by Ascent Software.

As metabolic activity is not necessarily proportional to proliferative activity, average cell numbers were determined by using Bürker counting chambers. Trypan Blue dye was used to exclude dead cells.

3.6 mRNA isolation

mRNA from HT29 cells was extracted. Quantitative (Nanodrop) and qualitative (Bioanalyzer Pico 600 chip; RIN >8 in all cases) analyses were performed.

Cell numbers ranged from 100,000 to 11,135,000 per well. The isolated mRNA concentration ranged from 8 to 256 ng/μL per sample. The isolated mRNAs were pooled and used in the NanoString assay.

3.7 Gene expression analyses (Nanostring, Taqman RT-PCR)

The custom mRNA Assay Evaluation panel containing our custom gene code set was designed by NanoString. Genes were selected to

establish an association among *HGFR*, *IGF1R* and *TLR9* signaling pathways, autophagy, apoptosis, cell proliferation, and cancer cell stemness.

For validating the NanoString gene expression analysis results, Taqman RT-PCR was also performed for selected genes (*mTOR*, *ATG16L1*, *LC3B*, *BCN1*, *HGFR*, *IGF1R*, *PI3KCA*, *STAT3*, *CD95*, and *TLR9*). Gene expression levels for each individual sample were normalized to *GAPDH* expression. Mean relative gene expression was determined and differences were calculated using the 2-ΔC(t) method. The whole cycle number was 45.

3.8 Immunocytochemistries

To detect HGFR, IGF1R, CD133, TLR9 and autophagy-associated ATG16L2, Beclin-1 and LC3 protein expression, HT29 cell smears were incubated with rabbit polyclonal anti-Met culture supernatant antibody (1:100, Clone: C-12), anti-IGF1R monoclonal antibody (Clone: 24-31; 1:50), anti-CD133/1-biotin antibody (1:100), mouse anti-human monoclonal anti-TLR9 antibody (20 μg/mL; Clone: 26C593.2) and anti-ATG16L1-, anti-BECN1- and anti-MAP1LC3B antibodies (1:200) at 37°C for 1 hour. Secondary immunodetection was performed using EnVision System Labeled Polymer–HRP K4001 (Anti-Mouse 1:1). A Liquid DAB+ Substrate Chromogen System was used to signal conversion. Cell smears were digitalized and analyzed using the CaseViewer software on a high-resolution PANNORAMIC 1000 FLASH DX instrument.

3.9 WES Simple and assessment of autophagic flux

For qualitative protein analyses, WES Simple was performed. A 12–230 kDa Separation Module was used for all the proteins (Phospho-mTOR Rabbit Ab; mTOR Rabbit mAb; Anti-SQSTM1/p62 Ab; ATG16L1 Rabbit mAb; Beclin-1 Rabbit mAb; LC3B XP Rabbit mAb; Anti-β-actin Mouse mAb; GAPDH Rabbit mAb) and either the Anti-Rabbit Detection Kit (ProteinSimple DM-001) or Anti-Mouse Detection Kit (ProteinSimple DM-002) was used, depending on the primary antibodies.

3.10 Cell counting and interpretation of immunoreactions

At 200x magnification, 10 fields of view and at least 100 cells (mainly 110 cells) per field of view were examined in a semiquantitative manner in each digitalized sample.

In the case of the *TLR9* and *HGFR* immune responses, weak, moderate and strong membrane staining and perinuclear cytoplasm staining were examined. In the case of the *IGF1R* immune response, weak, moderate and strong membrane staining and perinuclear cytoplasm staining were examined. As for autophagy, weak, moderate and strong *ATG16L1* and *Beclin-1* homogenous or spotted immunoreactions were detected in the cytoplasm. In the case of *LC3*, weak, moderate and strong punctuated or spotted cytoplasmic immunoreactions were observed.

3.11 Transmission electron microscopy for evaluation of autophagy

HT29 cells were fixed in 2% glutaraldehyde. The samples were post-fixed with 1% osmium tetroxide in 0.1M sodium-cacodylate buffer. Cells were centrifuged and embedded in 10% gelatin. The samples were embedded in Poly/Bed epoxy resin. Contrast staining of ultrathin sections (70-80 nm) with uranyl acetate and lead citrate was performed. JEM-1200EXII Transmission Electron Microscope was used to conduct ultrastructural analyses. The ultrastructure of the cells, as well as the average number of autophagic vacuoles were determined.

3.12 Semithin sections

Semithin sections were used for visualizing cell divisions. The sections were stained with toluidine blue. The average number of proliferating cells was counted.

3.13 Statistical analysis

Data on cell viability, cell number and proliferation were presented as means ± SD. The $\chi 2$-test and Student's t-test were used for statistical analyses. $p<0.05$ was considered statistically significant. In the case of immunocytochemistry, statistical analysis with one-way ANOVA and the Tukey HSD test was performed using R Core Team (2019). Regarding NanoString gene expression analysis, after importing RCC files to the nSolver Analysis Software, quality checking was performed. Then agglomerative cluster heat maps were

created. The Euclidean distance metric was used to calculate the distance between two samples (or genes) as the square root of the sum of squared differences in their log count values. The average linkage method was used to calculate the distance between two clusters. In the case of the WES Simple, the area of the tested proteins was multiplied by the values of the β-actin area for graphical representation.

4. RESULTS

mDNA achieved the most effective proliferation inhibition. fDNA increased tumor cell survival the most. After mDNA treatment, spheroid formation was observed in HT29 cells, despite the lowest tumor cell proliferation in this group. Spheroid cells showed prominin 1 (CD133) immunopositivity.

gDNA treatment resulted in an increase in *TLR9* gene expression and downregulation of *MyD88* and *TRAF6*. The mDNA treatment resulted in decreased expression of *TLR9*, *MyD88*, and *TRAF6* and increased expression of *IRAK2*, *NF-ΚB*, and *IL-8*. fDNA caused an increase in *TLR9*, *MyD88*, and *TRAF6* expressions with a decrease in *TNFα*, *IRAK2*, and *NF-ΚB*. The effect of m/fDNA was to increase *MyD88* and *TRAF6* expressions and decrease *TLR9*, *IRAK2*, *TNFα*, *IL-8*, and *NF-ΚB*. Overall, hypermethylation of self-DNA resulted in decreased *TLR9* expression, while DNA fragmentation increased *MyD88* and *TRAF6* expressions. DNA fragmentation abolished the stimulatory effect of hypermethylation on *IRAK2*, *NF-ΚB*, and *IL-8* expressions. For autophagy-associated genes, g- and fDNAs increased the expression of the *Beclin-1*, *ATG16L1*, and *LC3* genes. The m- and m/fDNAs caused weak expression of the same genes. Immunocytochemical analyses validated the gene expression results.

Compared to control, metabolically active HT29 cells, gDNA treatment resulted in more intense macroautophagy and sometimes dense vacuoles, which were more suggestive of late endosomes or autolysosomes. mDNA caused the cell structure to become disorganized, with chromatin condensation, blebbing, and nuclear fragmentation being the main signs of cells that were apoptotic. We also detected the presence of mitophagy. After fDNA treatment, we detected signs of lipophagy. In m/fDNA treatments, signs of

mitophagy were detected. All cell groups exhibited autophagy, but to varying degrees and quality. The mDNA treatment definitely induced apoptosis and activated mitophagy. Treatment of cells with fDNA enhanced cell survival, to which activated macroautophagy clearly contributed by preserving cellular fitness.

4.1 The interconnection of TLR9-mediated autophagy response and HGFR signaling

The gDC combination resulted in the most effective inhibition of cell proliferation with high metabolic activity. The mDC combination was the only one that reduced the increased metabolic activity caused by mDNA. For the mOD treatment combination, cell proliferation increased with significantly reduced metabolic activity.

Regarding canonical and non-canonical *HGFR* signaling pathways, gD treatment increased *STAT3*, *CD95*, and *PI3K* gene expression, with a decrease in *HGFR* expression. fD increased *HGFR* and decreased *STAT3* and *PI3K*, while mDNA+D increased *STAT3* and *HGFR* and decreased *PI3K* and *CD95* gene expression. Treatment of different cfDNAs with O+D together decreased the expression of both canonical and non-canonical *HGFR* signal transduction genes. It was found that gD inhibited, while D/C+fDNA and mC strongly increased the expression of HGFR signaling genes. The combined effects of HGFR inhibition and modified cfDNA treatment increased the expression of *ATG16L1*, *LC3*, *Beclin-1*, and *ULK1*, except for fDNA-Beclin-1 and mDNA-ULK1, respectively. Combining g- or mDNAs with D+O treatment resulted in the downregulation of all autophagy-associated genes. fDNA, on the other hand, exhibited the opposite effect. Individual cfDNAs in combination with D+C treatment increased the expression of all autophagy-associated genes. Taqman RT-PCR confirmed the NanoString results.

The immunocytochemical results of TLR9, HGFR, ATG16L1, Beclin-1, and LC3B also validated the gene expression results at the protein level. Changes in LC3B protein levels were consistent with the changes observed in gene expression and immunocytochemistry assays. Quantitative analysis of LC3B and p62 proteins confirmed that combinations of certain cfDNA treatments with HGFR inhibitors enhance the autophagy inhibitory effect of chloroquine. Indeed, inhibition of autophagy resulted in the accumulation of these proteins by reducing the degradation of LC3B and p62 proteins.

HGFR inhibitor experiments revealed AVs indicative of macroautophagy in control (3±1/cells) and chloroquine-treated (4±1.5) HT29 cells. In D and O cells, the frequency of AVs was higher (D: 6±1.8; O: 7±1.4). Treatments O, D, or C combined with gDNA resulted in more intense macroautophagy (O: 9±1.2; D: 7±2; C: 7±1.6; gDNA: 6±2). The treatment with fDNA resulted in the appearance of MVBs in addition to AVs (5±1.8). The fO combination increased the number of AVs (12±2), while fD (6±1.4) and fC (4±2.3) did not significantly change the number of AVs, while the cell structure was disorganized. Incubation with mDNA (7±1.3) also resulted in disorganization of cell structure, chromatin condensation, and blebbing. The mC combination resulted in the appearance of (5±1.6) MVBs in addition to AVs. However, the mD treatment (7±1.4) enhanced cell survival, to which activated cell-protective macroautophagy obviously contributed. The mO resulted in the least AV appearance (2±1.3). To investigate whether the reduction in cell number after cfDNA and inhibitor treatments was due to low proliferative activity or increased cell death, we also examined semithin sections. In the case of incubation with g-, f-, or mDNAs, the incidence of proliferation was proportional to the cell numbers obtained. The gDC combination resulted in significantly reduced proliferation activity, whereas mOD resulted in increased proliferation activity.

4.2 The interconnection of TLR9-mediated autophagy response and IGF1R signaling

All treatment groups, except the gOP combination, significantly increased the metabolic activity of HT29 cells. Cells treated with gDNA exhibited the lowest cell proliferation. Combination of gDNA with O, P, or C caused effective inhibition of proliferation with increased metabolic activity. The combination of gOP restored the proliferative activity of HT29 cells to levels close to those of the control group.

With respect to *IGF1R* signaling, gP treatment caused a small increase in *IGF1R* expression without a significant change in *MAPK*, *PI3K*, and *Akt* expression. The gC, gO, and gOC combinations resulted in increased expression of *IGF1R*-associated genes. The gPC treatment resulted in the greatest gene expression enhancement for *TLR9* signaling (*MyD88*, *NF-ΚB*), autophagy-associated (*ATG16L1*,

Beclin-1, *LC3*, *ULK1*, *Ambra-1*), autophagy-inhibitory/anti-apoptotic (*PI3K*, *Akt*, *mTOR*), and autophagy-activating/pro-apoptotic (*MAPK*, *AMPK*, *Bax*) genes. In contrast, gOP treatment caused a general inhibition of the expression of the genes tested, with the exception of *TLR9* and *Bcl-2*. CD133 mRNA expression was increased for gDNA, P, C, O, and gPC combinations.

Immunocytochemical analyses of IGF1R, ATG16L1, Beclin-1, LC3B, and CD133 confirmed the gene expression results. The gP treatment resulted in inhibition of autophagy initiation through decreased gene expression of PI3K, Akt, AMPK, and mTOR. Autophagy inhibition leads to activation of mTOR. Qualitative protein expression assays of the control, gDNA, and gP HT29 groups showed that mTOR, phospho-mTOR, and autophagy-associated protein expression and phospho-mTOR activity were consistent with the gene expression results.

The frequency of AVs was higher in O (7±1.4) cells and lower in P (0.5±0.5) compared to controls. The gO (10±2.2) and gC (5±1.5) treatments resulted in the presence of intense autophagy with chromatin condensation, blebbing, and disorganized cellular structure. Combinations of gP (2±1.5) and gOP (3±1) showed low numbers of AVs. In contrast, gPC caused intense macroautophagy (11±2.6). The gP combination also showed MVBs. In semithin sections, significantly decreased proliferative activity was observed in the gP and gPC groups, whereas increased proliferative activity was observed in the gOP combination.

5. CONCLUSIONS

The mDNA treatment has proapoptotic and proliferation-inhibitory effects in HT29 cells. However, DNA fragmentation (f/mDNA) reduces the antiproliferative effect of hypermethylation. Both TLR9-dependent and TLR9-independent molecular mechanisms are involved in this phenomenon.

Treatment with fDNA increases the viability and proliferation of HT29 cells and decreases cell death. DNA fragmentation is also linked to higher levels of *TLR9*, *MyD88*, and *TRAF6* expression and lower levels of *TNFα*, *IRAK2*, and *NF-ΚB* expression.

Structural modifications of cfDNA (fragment length, methylation status) profoundly affect the extent and ultimate biological impact of *TLR9*-mediated signaling pathways and *TLR9*-dependent autophagy activation.

Inhibition of HGFR blocked the cell proliferation-reducing effects of g- and mDNA. Increased expression of *MyD88* and *caspase-3* stimulated HT29 cell proliferation, suggesting that fDNA treatment inhibits apoptosis-induced "compensatory" cell proliferation. HGFR inhibitor treatment can enhance this phenomenon by increasing *MyD88* expression. The accumulation of p62 and LC3B proteins suggests an impairment of autophagy, which may also explain this observation.

The gDC combination was the strongest inhibitor of HT29 cell proliferation. The inhibitory effect of LC3B counterbalanced the cell proliferation-stimulatory effect of increased *STAT3* expression in this case. This underlines the role of the *HGFR-mTOR-ULK1* molecular cascade in HGFR inhibitor-mediated autophagy.

The mOD combination treatment resulted in the highest HT29 cell proliferation. This was due to a concomitant downregulation of canonical and non-canonical *HGFR* signaling pathways and autophagy-associated genes.

The presence of MVBs was associated with increased expression of the *Beclin-1* and *PI3K* genes. This suggests a coupling of the autophagosome and MVB pathways, i.e., exosome biogenesis and autophagy are involved together in maintaining cell homeostasis and enhancing their stress tolerance.

Simultaneously administering tumor-derived cfDNA and inhibiting IGF1R in HT29 cells demonstrates an inhibitory effect on proliferation. This can be counteracted by a parallel inhibition of *TLR9* signaling.

The effect of *IGF1R*-associated autophagy mechanisms on cell proliferation by cfDNA and inhibitor combinations is context-dependent. However, the cell-protective effect of autophagy may even be detrimental, as it favors the survival of some CD133+ (stem-like) cancer cells.

6. BIBLIOGRAPHY OF THE CANDIDATE'S PUBLICATIONS

Publications / abstracts related to PhD dissertation

(subtotal IF: 11.2)

1. Bohusné Barta Bettina, Simon Ágnes, Nagy Lőrinc, Dankó Titanilla, Raffay Regina Eszter, Petővári Gábor, Zsiros Viktória, Sebestyén Anna, Sipos Ferenc, Műzes Györgyi. Survival of HT29 cancer cells is influenced by hepatocyte growth factor receptor inhibition through modulation of self-DNA-triggered TLR9-dependent autophagy response. PLOS ONE 2022; 17(5): e0268217. **IF: 3.7 (Q1)**

2. Sipos Ferenc **/ Bohusné Barta Bettina**, Simon Ágnes, Nagy Lőrinc, Dankó Titanilla, Raffay Regina Eszter, Petővári Gábor, Zsiros Viktória, Wichmann Barnabás, Sebestyén Anna, Műzes Györgyi. Survival of HT29 cancer cells is affected by IGF1R inhibition via modulation of self-DNA-triggered TLR9 signaling and the autophagy response. PATHOLOGY AND ONCOLOGY RESEARCH 2022; 28: 1610322. **IF: 2.8 (Q2)**

3. Műzes Györgyi, **Bohusné Barta Bettina**, Szabó Orsolya, Horgas Vanessza, Sipos Ferenc. Cell-Free DNA in the Pathogenesis and Therapy of Non-Infectious Inflammations and Tumors. BIOMEDICINES 2022; 10(11): 2853. **IF: 4.7 (Q1)**

4. Sipos Ferenc, **Bohusné Barta Bettina**, Dankó Titanilla, Sebestyén Anna, Zsiros Viktória, Műzes Györgyi. Relation of IGF1R inhibition to TLR9- and autophagy signaling in HT29 cancer cells. ALLERGY: EUROPEAN JOURNAL OF ALLERGY AND CLINICAL IMMUNOLOGY 2023; 78(S111): 281.

5. Sipos Ferenc, Nagy Lőrinc, **Barta Bettina**, Simon Ágnes, Dankó Titanilla, Sebestyén Anna, L. Kiss Anna, Műzes Györgyi. Modulation of TLR9-dependent autophagy response via inhibition of c-Met signaling influences the survival of HT29 cancer cells. ANNALS OF ONCOLOGY 2019; 30(Suppl.5): 807.

6. Nagy Lőrinc, **Barta Bettina**, Simon Ágnes, Sebestyén Anna, Dankó Titanilla, Raffay Regina, Műzes Györgyi, Sipos Ferenc. Structurally modified forms of genomial self-DNA influence appreciably in vitro HT29 cancer cell survival through TLR9-signaling and modulation of autophagic response. In: 9th Lower Saxony International Summer Academy (LISA) in Immunology - Abstract Book 2019; p. e58.

Additional papers / abstracts not related to the PhD thesis

(subtotal IF: 5.3)

1. Műzes Györgyi, **Bohusné Barta Bettina**, Sipos Ferenc. Colitis and Colorectal Carcinogenesis: The Focus on Isolated Lymphoid Follicles. BIOMEDICINES 2022; 10(2): 226. **IF: 4.7 (Q1)**

2. Bohusné Barta Bettina; Sipos Ferenc, Műzes Györgyi. Az intestinalis bojtos sejtek sajátosságai és szerepük a gyulladásos bélbetegség és a colorectalis carcinoma patomechanizmusában. ORVOSI HETILAP 2023; 164(44): 1727-1735. **IF: 0.6 (Q4)**