FINAL REPORT

Role of bile acids in cancer patology

1. INTRODUCTION

The human body harbors numerous bacteria in the cavities and on the body surface. The ensemble of these microbes is referred as the microbiota and its collective genome as the microbiome. Alterations of the microbiome are associated with certain cancers. Although, the microbiota may have a widespread role in carcinogenesis, the number of directly tumorigenic bacteria is extremely small [1]. It seems more likely that pathological changes in the microbiota/microbiome (dysbiosis) determine susceptibility to the disease or influence the progression of the disease [2]. Most human malignancies affect those organs that are directly in contact with microbes such as the urinary tract [3], skin [4], immune system [5], and the colon [6]. Little is known about the interconnection between microbiome and tumors that are not in direct contact with the microbiome [7, 8].
Bacteria secrete metabolites that either exert their effects locally, in a paracrine fashion, or enter the circulation and modulate distantly located cancer cells. For paracrine metabolites, the best examples are those involved in the carcinogenesis of colorectal cancer [9]. The metabolites that act on distantly located cancer cells have similar properties as classical human hormones; they are produced in a "gland", i.e., the microbiome, and then transported to distant organs, where they regulate physiology and behavior [10]. Cadaverine [11], lithocholic acid [12], deoxycholic acid [13], and short chain fatty acids [14] have been identified as hormone-like metabolites. These metabolites have pleiotropic effects and modulate multiple cancer hallmarks simultaneously. The metabolites can inhibit proliferation, decrease epithelial-to-mesenchymal transition, reduce tumor metastasis, decrease cell migration and transmigration, induce antitumor immunity, rearrange cellular metabolism, induce senescence, and reduce cancer cell stemness [11-16].

2. AIMS

Our aim was to characterize the effect of bile acids in different tumor types focusing on breast cancer, ovarian, pancreatic adenocarcinoma and glioblastoma multiforme. We tried to answered the following questions: 1. How bile acids affect the classical hallmarks of cancer? 2. Could bile acids revert or enhance the Warburg-type metabolism and influence mitochondrial activity? 3. Could bile acids modify the oxidative stress responses? 4. Through which bile acid receptor and signaling pathways exert bile acids their effect?

3. METHODS

The following cancer cell lines were used: breast cancer- MCF7, 4T1, SKBR3; pancreas adenocarcinoma - Capan2, BXCP3, PancTu1; ovarian cancer – A2780; glioblastoma multiforme– T98, U251.
In animal study we used 4T1 breast cancer cells that were grafted into immunocompetent Balb/c mice.
- **Cellular proliferation**: Proliferation of cells were characterized by short term assays such as Sulphorhodamine B assay and/or DAPI staining of cells monitored by High Content Screening (HCS) method. Long term proliferation was studied by colony forming assay. Acute toxicity was measured in propidium-iodide uptake assays.
- **EMT, cell movement and cell morphology**: The expression of EMT marker genes was assessed by RT-qPCR and Western blot technique and/or immunocytochemistry. Morphology of the cytoskeleton system (F-actin) was assessed by Texas Red-X phalloidin staining followed by confocal microscopy. Cell adhesion and cell-to-cell connection were determined using the

Electric Cell-substrate Impedance Sensing (ECIS) system. Cell migration and invasion were determined using Boyden chambers and/or scratch assay. In scratch assays cell densities were monitored using JuLi Br Live cell movie analyzer.

- **Mitochondrial functions**: Mitochondrial biogenesis was characterized by the measurement of the expression of mitochondrial genes (e.g. genes participating in oxidative phosphorylation (OXPHOS) system, citric acid cycle and/or are regulator proteins) by RT-qPCR and Western blot. Mitochondrial activity was characterized by measuring mitochondrial oxygen consumption using Seahorse XF96 instrument and mitochondrial membrane potential using DioC6 fluorescence. The activity of energy sensors were monitored through the phosphorylation of their target proteins using Western blot technique. Glycolysis was determined by Seahorse XF96 instrument using the assay depends on lactate production. Metabolomics were performed as in [12].
- **Oxidative stress:** Expression level of NRF2, several antioxidants, NRF2 target genes and ROS generating enzymes were measured by RT-qPCR and/ or Western blot technique. Oxidative stress was assessed by superoxide production (hydroethidine fluorescence), iNOS expression (RT-qPCR, Western blot) and nitrothyrosine expression (Western blot). Lipid peroxidation was determined by TBARS assay and measurement of 4-HNE expression.
- **Receptor analysis:** We used bile acid receptor antagonists and specific siRNAs (Silencer Select siRNAs) to identify receptor(s) responsible for the effects of bile acids. For the silencing of the key receptor(s) we used transient transfection. Transfection efficiency was monitored by qPCR and/or Western blot technique.
- **In silico screening**: The kmplot.com database was used to study the link between gene expression levels and cancer survival in humans. The most common mutations in different cancer types were obtained from the intogen database. Gene expression profiles were checked from the Gene Expression Omnibus database. Several signaling pathways, components and interactions were studied from KEGG pathway and PANTHER database.
- **Animal studies**: Experimental animals were female BALB/c animals between 8-10 weeks of age. Animals were bred in the “specific pathogen free” zone of the Animal Facility at the University of Debrecen, and kept in the “minimal disease” disease zone during the experiment. Tumor cell injection: In case of breast cancer 4T1 mouse cells were suspended ($2x10^6$/mL) in PBS-matrigel at 1:1 ratio. From this suspension female BALB/c mice received 50 µL injections to their 2nd inguinal fat pads on both sides (10^5 cells/injection). Bile acid treatment: Animals received daily oral bile acid treatments. Bile acid stock were prepared in 96% ethanol at 100x concentration (7.5 mM). Ethanol vehicle (1% in PBS) was prepared and diluted similarly. Animals received a daily oral dose of 200 µL/30 g bodyweight from the bile acid solution or the vehicle. During autopsy tumors will be visually assessed and scored based on their infiltration rate into surrounding tissues. From tumor samples we isolated RNA (using Trizol reagent) and protein.
- **Human studies:** Human fecal samples were collected from healthy and breast cancer patients by James J. Goedert laboratory at the National Cancer Institute (NCI). DNA isolated from these human fecal samples were used to determine the abundance of the bacterial genes (bai genes) coding key enzyme of bile acid biosynthesis in breast cancer patients. Bai gene specific primers were designed using Primer3 software. For the assessement of the expression of the bai genes ~10 ng of DNA was used for qPCR reactions. Specificity of the qPCR reactions was verified by sequencing PCR products with the primers used for the amplification. From serum samples bile acid profile were assessed as in [12].

4. RESULTS

4.1. Role of bile acids in breast cancer

4.1.1. Lithocholic acid reduces the aggressiveness of breast cancer

In our first study we investigated three secondary bile acids, lithocholic acid (LCA), deoxycholic acid (DCA) and ursodeoxycholic acid (UDCA) in concentrations corresponding to their normal (reference) concentrations in human serum and breast tissue (10 nM–10μM). In short term proliferation assay we found that LCA reduced cellular proliferation of MCF7, SKBR3 and 4T1 breast cancer cells but did not affect primary fibroblasts. Other secondary bile acids, such as DCA or UDCA were without effect on breast cancer cells. The percent of propidium-iodide positive cells did not change upon LCA treatment suggesting that LCA did not induce cell death. Further, we tested the cytostatic property of LCA in mice that were grafted with 4T1 cells and were treated with LCA (15 nmol LCA p.o. q.d.) or vehicle for 18 days. The infiltration capacity of the primary tumor to the surrounding tissues markedly decreased upon LCA treatment and the number of the metastases was also lower in the LCA-treated group.

4.1.2. Lithocholic acid modulates classical cancer hallmarks in breast cancer

We investigated how LCA modulates the different features of breast cancer through assessing classical hallmarks of cancer. Our results showed that LCA treatment reverted epithelial mesenchymal transition (EMT), improved cell-to-cell connections, inhibited β-catenin signaling both in cell lines and in vivo and reduced cell movement in a scratch assay. In addition, we found lower VEGF mRNA expression and higher number of tumor infiltrating lymphocytes (TILs) in LCA-treated mice.

Further we assessed LCA-induced changes in cellular metabolism. LCA treatment induced glycolysis (extracellular acidification rate - ECAR) and mitochondrial respiration (oxygen consumption rate - OCR). In line with these observations, LCA-induced intracellular lactate and citrate levels and induced the expression of a set of OXPHOS genes in breast cancer cells. In pulse-chase metabolomics experiments breast cancer cells were treated with 300 nM LCA. When cells were charged with ^{13}C-acetate, a metabolite that can fuel the TCA cycle, LCA treatment enhanced the incorporation of ^{13}C into succinate and malate suggesting increased flux through the TCA cycle. Then cells were charged with ^{13}C-glucose from which ^{13}C atoms must enter glycolysis to subsequently feed the TCA cycle or form lactate. LCA treatment enhanced the amount of ^{13}C-labelled citrate and lactate in MCF7 cells and the amount of ^{13}C-labelled succinate and lactate in 4T1 cells. In line with these observations, the ratio between ^{13}C-citrate and ^{13}C-lactate or between ^{13}C-succinate and ^{13}C-lactate increased, providing further evidence towards mitochondrial dominance of the LCA-induced metabolic switch. All these results suggest that LCA treatment induced the TCA cycle and oxidative phosphorylation in breast cancer cells. LCA also induced the expression of positive regulators of mitochondrial oxidative phosphorylation such as FOXO1, PGC-1β, and enhanced the expression and nuclear translocation of NRF1. LCA also induced AMPK activity.

Further, we aimed to identify the LCA receptor(s) responsible for the above effects of LCA. At first we used pharmacological inhibitors designed to inhibit different LCA receptors. We used NF449, U73343 to block TGR5; CINPA1 to block CAR, DY268 to block FXR and GSK2033 to blok LXR receptors. Breast cancer cells were treated with LCA together with vehicle or pharmacological agents blocking the potential LCA receptors. LCA-mediated reduction in cell proliferation was efficiently blocked by CINPA1, NF449 and U73343; other inhibitors were ineffective. NF449 and U73343, unlike CINPA1, efficiently blocked LCA-induced morphological changes in breast cancer cells. Therefore, we transiently silenced TGR5 in MCF7 cells to provide direct evidence for the involvement of TGR5. Silencing of TGR5 efficiently blocked LCA-induced morphological changes and blocked the LCA-induced increases in the mRNA expression of mitochondrial markers and markers of AMPK activation. All these results suggest that the effect of LCA is mediated through TGR5 receptor in breast cancer cells.

4.1.3. Lithocholic acid biosynthesis is suppressed in early phases of human breast cancer

Further we investigated how bile acid and LCA metabolism relates to breast cancer in humans. The enzymes involved in the 7-dehydroxylation of bile acids are organized into one operon called the bile acid inducible (bai) operon, wherein the baiH ORF codes for 7-HSDH, a key enzyme of LCA synthesis in most bacterial species. We identified the baiH ORF of anaerobic, Gram positive and Gram negative species and measured the abundance of the baiH DNA in fecal DNA samples using qPCR assays. When all patients were compared to healthy controls the abundance of baiH of Clostridium sordelli, Staphylococcus haemolyticus, Escherichia coli and Pseudomonas putida was lower in breast cancer patients. A more pronounced decrease in the abundance of the baiH of Bacteroides thetaiotaomicron, Clostridium sordelli, Staphylococcus haemolyticus, Escherichia coli and Pseudomonas putida were observed in stage 0 and stage 1 patients.

We found that total bile acid, CDCA and LCA levels were reduced in serum from breast cancer patients as compared to age and sex matched healthy individuals. We also found a decrease in the LCA/CDCA ratio in breast cancer patients compared to healthy individuals, and this decrease that was more marked when only stage 1 patients were assessed.

Taken together, the bacterial LCA biosynthesis machinery in the intestine is downregulated in breast cancer patients that is very pronounced in the early phase of the disease. Lower capacity to synthesize LCA then contributes to lower LCA levels.

4.1.4. Lithocholic acid induced oxidative stress by reducing NRF2 expression in breast cancer

In our second study connected to breast cancer we assessed the involvement of LCA in oxidative stress responses in breast cancer. We found that LCA treatment could influence the expression of key elements in the antioxidant NRF2/KEAP1 pathway. LCA decreased NRF2 protein level while upregulating protein expression of the NRF2 repressor, KEAP1. Other secondary bile acids (DCA and UDCA) had no effect on the key components of NRF2/Keap pathway. Pharmacological activation of NRF2 by RA839 abolished the anti-proliferative effects of LCA. These results show that decreased NRF2 expression played a key role in eliciting the cytostatic effects of LCA.

Oxidative stress is an imbalance between antioxidant and prooxidant genes. In our further experiments we investigated how LCA modulates different oxidative stress markers in breast cancer cells. We also confirmed that LCA reduced protein level of other antioxidant gene, GPX3 and increased the mRNA expression of ROS producing enzyme, NOX4. We detected increased lipid and protein oxidation using TBARS assay, 4-hydroxynonenal adducts and protein carbonylation upon LCA treatment. We also assessed the expression of iNOS, one of the important source of nitric oxide in cells. iNOS protein level was higher in LCA treated breast cancer cells compared to control. High level of NO have the opportunity to react with superoxide (O_2^-) leading to peroxynitrite ($ONOO^-$) formation. LCA induced the level of nitrotyrosine which is a good marker for indirect detection of peroxynitrite.

The activation of NRF2 by RA839 or tBHQ prevented both increases in TBARS and 4HNE and decreases in iNOS expression when we applied in combination with LCA. These results suggest that the above mentioned effects of LCA is regulated by NRF2. Treatment of breast cells with synthetic antioxidants, glutathione (GSH) and N-acetyl cysteine (NAC) together with LCA verified that these antioxidants are able to change LCA-induced antiproliferative response similarly to the NRF2 activation.

LCA did not modulate the expression of NRF2, KEAP1, or 4HNE in primary, non-transformed human fibroblast suggesting that the effect of LCA is specific to breast cancer cells.

Further, we aimed to identify the receptors responsible for the effects of LCA. First, we used pharmacological agents designed to inhibit LCA receptors, including CINPA1 to inhibit the constitutive androstane (CAR) receptor, DY268 to inhibit the farnesyl-X receptor (FXR), GSK2033 to inhibit the liver X receptor (LXR), and NF449, a Gsα-selective antagonist that can inhibit the downstream signaling of the TGR5 receptor. LCA-mediated reduction in NRF2

protein expression was blocked by NF449 and CINPA1, while the other inhibitors (GSK2033 and DY268) were ineffective.

The pharmacological experiments then were complemented by siRNA depletion experiments. Other possible LCA receptors, including the vitamin D receptor (VDR) and the pregnane X receptor (PXR), were also assessed. We silenced, TGR5, CAR, VDR, and PXR in MCF7 cells and found that silencing of TGR5 and CAR efficiently blocked the LCA-induced decreases in NRF2 protein, similarly to the pharmacological agents. Silencing of VDR and PXR receptor had no effect on LCA-mediated reduction of NRF2 protein levels. TGR5 and CAR silencing also blocked the LCA-induced increases in iNOS. These data indicate that LCA exerts its effects through the TGR5 receptor and CAR receptor.

In the tumors of control and LCA-treated mice we measured the expression of anti and pro-oxidant genes. LCA tratment reduced the expression of NRF2 and a set of antioxidant genes: catalase (CAT), glutamate-cysteine ligase catalytic subunit (GCLC), glutathione peroxidase 2 (GPX2), glutathione peroxidase 3 (GPX3), heme oxygenase 1 (HMOX1), inducible NO synthase (iNOS), NADPH oxidase 4 (NOX4), NAD(P)H quinone dehydrogenase 1 (NQO1), nuclear factor, erythroid 2 like 2 (NRF2), superoxide dismutase 1 (SOD1), superoxide dismutase 2 (SOD2), superoxide dismutase 3 (SOD3).

4.1.5. Elements of the lithocholic acid-induced anticancer pathway show correlation with stage, grade, and receptor status of the disease

Further we measured the expression of the LCA-elicited oxidative/nitrosative stress markers (TGR5, iNOS, and 4HNE) using a tissue microarray (TMA) made up of tumor samples from 88 breast cancer patients.

First, we stratified patients for the TMA, based on the disease stage from stage I to stage IV, based on the primary tumor size, the lymph node involvement, and distant metastasis. Levels of iNOS and 4HNE decreased in stage II and stage III patients compared to stage I patients and further decreased in stage IV patients.

Then we stratified patients based on the pathological grade (Nottingham grade) of the disease. 4HNE expression significantly decreased in grade II and grade III patients compared to grade I patients.

We also stratified patients as triple negative (TNBC; ER- PR- HER2-) or ER+ cases. The expression of TGR5, iNOS and 4HNE decreased in TNBC cases as compared to ER+ cases. In line with that, higher expression of CAR, KEAP1, iNOS, nNOS and NOX4, or the lower expression of NRF2 was associated with better survival when we assessed all patients or ER+ positive cases, but not in TNBC cases examined in kmplot database.

Finally, we grouped patients as a function of the mitosis score. Staining for 4HNE, the most direct indicator of tissue oxidative stress, decreased as mitosis score increased. Furthermore, 4HNE staining showed a strong negative correlation with the mitosis index.

All these results suggest that the LCA-oxidative stress protective pathway is lost as breast cancer progresses, and the loss correlates with poor prognosis.

4.2. Role of bile acids in pancreatic adenocarcinoma

We studied the effects of secondary bile acids in Capan2 pancreas adenocarcinoma cell line. We treated Capan2 cells with LCA, DCA and UDCA in different concentrations for two days. LCA (0.01 uM and 0.03 uM) and UDCA (0.3 uM) significantly decreased Capan2 cell proliferation as measured in SRB assays. DCA was without effect in this cell line. LCA and UDCA inhibited cellular proliferation implicating modulation of the epithelial- mesenchymal transition (EMT). We also observed that UDCA improved cell-to-cell connection, as reflected by epithelial-like morphology in cells (Phalloidin-Texas Red staining). LCA and UDCA significantly decreased the protein expression of SNAIL, SLUG and β-CATENIN mesenchymal

markers. Both bile acids induced the protein expression of epithelial marker, ZO-1. All these results suggest that bile acids reverted EMT process in Capan2 adenocarcinoma cell line. In line with these observations, LCA and UDCA- treated cells migrated slowly in Matrigel coated Boyden chamber compared to untreated cells.
We also assessed different oxidative stress markers to explore the redox status of bile acid treated Capan2 cells. UDCA and LCA treatment reduced the expression of nuclear factor erythroid 2-related factor 2 (NRF2), which is a transcription factor responsible for the expression of antioxidant enzymes. In parallel, we observed the enhanced expression of inducible nitric oxide synthase (iNOS), an enzyme promoting nitrosative stress. Bile acids did not influence lipid peroxidation in Capan2 cells.
Further, we assessed markers of cellular energy stress (pACC, pAMPK, FOXO1, and PGC1β). Our results show that UDCA increased the expression of positive regulators of mitochondrial oxidation, FOXO1, PGC-1β and induced AMPK activity (pACC and pAMPK protein expression). Nuclear receptors, FXR, PXR, LXR, CAR, VDR and G-protein coupled TGR5 receptor serves as receptors for secondary bile acids. First, we used pharmacological agents designed to inhibit bile acid receptors, including CINPA1 to inhibit the constitutive androstane (CAR) receptor, DY268 to inhibit the farnesyl-X receptor (FXR), GSK2033 to inhibit the liver X receptor (LXR), and NF449, a Gsα-selective antagonist that can inhibit the downstream signaling of the TGR5 receptor. UDCA-mediated reduction in Matrigel migrating cells was blocked by CINPA1, while LCA-mediated reduction in Matrigel migrating cells was blocked by CINPA1 and DY268, the other inhibitors were ineffective. To verify pharmacalogical expreriments, we started to silence TGR5, CAR, FXR, LXR, VDR, PXR receptors using siRNAs in Capan2 cells. The efficiency of silencing was verified in qPCR experiment. Next, we would like to assess the expression of EMT, oxidative stress and energy sensor markers after silencing of different bile acid receptors to verify their exact role in bile acid mediated changes in pancreatic adenocarcinoma cells. We also verified some characteristic effects of LCA and UDCA in two other human pancreatic adenocarcinoma cell lines, BXPC3 and PancTu1.

4.3. Role of bile acids in ovarian adenocarcinoma

We assessed whether bile acids treatment could influence the proliferation of A2780 ovarian cancer cell line. UDCA slowed down the cellular proliferation while DCA induced proliferation of A2780 ovarian cancer cells monitored by high content screening (HCS) method and by measuring the expression level of pP70S6K proliferation marker gene. LCA was without effect on A2780 cells. UDCA treatment increased while DCA decreased mitochondrial membrane potential using DioC6 fluorescence. In line with these observations, UDCA induced the expression of a several mitochondrial genes, such as ATP5g1, Cytochrome C, Fumarase and IDH2 in A2780 cells. DCA decreased the mRNA expression level of Cytochrome C while the expression level of other examined genes involving in mitochondrial biogenesis were unchanged. In Seahorse experiment we found that UDCA induced mitochondrial oxidation (increased OCR/ECAR ratio) while DCA reduced mitochondrial activity (decreased OCR/ECAR ratio). We also assessed components of the cellular energy sensor pathway and mitochondrial transcriptional regulators. We found that UDCA induced the expression of pAMPK and pACC at protein level showing induction of AMPK activity. DCA has an opposite effect on AMPK activity. The expression of positive regulators, FOXO1, PGC-1β and NRF1 did not change significantly in A2780 pancreas adenocarcinoma cell line.
Based on these results, we expected that UDCA support mesenchymal-epithelial transition (MET) process. Interestingly, our further results showed that UDCA increased the RNA and protein expression of several mesenchymal markers including β-CATENIN, TWIST1, SNAIL, TCF7L2 in A2780 cells. Migration of A2780 cells through the matrigel layer was enhanced by UDCA in Boyden chamber. Thus, UDCA increased the invasiveness of the cells compared to the control sample.
The discrepancy in the effects of UDCA on cellular metabolism and EMT process need to be further investigated.

4.4. Role of bile acids in glioblastoma multiforme

We treated U251 and T98 glioblastoma cell lines with secondary bile acids in concentrations corresponding to their reference concentrations in human serum (LCA > 0,1 μM; DCA 0,3-0,7 μM; UDCA 0,1-0,3 μM) for 2 days. LCA increased proliferation of glioblastoma cells using short term proliferation assay (SRB assay). DCA and UDCA had no effects on glioblastoma cell proliferation. Further we found that LCA enhanced the protein expression of pP70S6K which is a good proliferation marker. We verified the pro-proliferative effect of LCA on U251 cells using longer colony forming assay. T98 cells could not form colonies. Next, we assessed LCA induced changes in cellular metabolism. LCA reduced the expression of positive regulator of mitochondrial oxidative phosphorylation PGC-1α, PGC-1β and FOXO1. We also observed LCA-mediated reduction of AMPK activity (decreased pACC and pAMPK levels) in glioblastoma cells. mRNA expression level of mesenchymal markers including SNAIL, TWIST1, TCF7L2, β-CATENIN and angiogenic factor VEGFA increased after LCA treatment. LCA also induced the protein level of β-CATENIN in glioblastoma cells. Further we found that LCA induced the expression of NRF2 antioxidant protein and reduced iNOS expression.

5. CONCLUSION

In our first two studies we provided a mechanistic relationship between the gut microbiome and breast cancer. Bacteria can secrete metabolites that either exert their effects locally or through the circulation reach distantly located cancer cells and influence their behavior. We used cellular models (4T1, MCF7, SKBR3) and mouse model (4T1 grafted Balb/c mice) of breast cancer, and samples (serum, fecal DNA, breast tissue) from breast cancer and healthy patients to study the effects of LCA which is a hormone-like metabolite specifically synthesized by intestinal microbes.

LCA inhibited breast cancer cell proliferation, decreased epithelial-to-mesenchymal transition, reduced tumor metastasis, induced antitumor immunity and rearranged cellular metabolism. LCA increased oxidative and nitrosative stress in breast cancer cells through modulation the expression level of antioxidant (NRF2, GPX3) and pro-oxidant (iNOS, NOX4) genes *in vitro* and *in vivo.* The imbalance between the pro- and antioxidant enzymes increased cytostatic effects of LCA via increased levels of lipid and protein oxidation. We identified Takeda G-protein coupled receptor (TGR5) and constitutive androstane receptor (CAR) as a receptors of LCA that mediates these anticancer effects. In humans we showed that early stage breast cancer patients had reduced serum LCA levels and reduced abundance of the baiH (the key enzyme in LCA generation) gene in fecal DNA samples. The expression of key components of the LCA-elicited cytostatic pathway (iNOS and 4HNE) gradually decreased as the breast cancer stage advanced. The level of lipid peroxidation in tumors negatively correlated with the mitotic index. The overexpression of iNOS, nNOS, CAR, KEAP1, NOX4, and TGR5 or the downregulation of NRF2 correlated with better survival in breast cancer patients, except for triple negative cases.

All these results suggest that LCA has pleiotropic effects and modulate multiple cancer hallmarks in breast cancer. Bacterial LCA production is reduced in early breast cancer suggesting the involvement of low LCA levels in the pathogenesis of breast cancer. The LCA–oxidative stress protective pathway is lost as breast cancer progresses, and the loss correlates with poor prognosis.

We also studied the effect of secondary bile acids in different tumor types focusing on ovarian, pancreatic adenocarcinomas and glioblastoma multiforme.

Our results showed that the effect of bile acids are different in different cancer types, and bile acids act through different bile acid receptors in cancers. In contrast to breast cancer LCA enhances neoplastic processes in glioblastoma cells. LCA and UDCA have protective effects in pancreatic adenocarcinoma, both bile acids inhibit neoplastic processes. The effect of bile acids in ovarian adenocarcinoma cells is variable.

6. REFERENCES

1. Martel, C, et al., Global burden of cancers attributable to infections in 2008: a review and synthetic analysis. Lancet Oncol., 2012. 13(6): p. 607-15
2. Garrett, W.S. (2015). Cancer and the microbiota. Science 348, 80-86.
3. Yu, H., Meng, H., Zhou, F., Ni, X., Shen, S., and Das, U. N. (2015) Urinary microbiota in patients with prostate cancer and benign prostatic hyperplasia. Archives of medical science : AMS 11, 385-394
4. Yu, Y., Champer, J., Beynet, D., Kim, J., and Friedman, A. J. (2015) The role of the cutaneous microbiome in skin cancer: lessons learned from the gut. Journal of drugs in dermatology : JDD 14, 461-465
5. Goldszmid, R. S., Dzutsev, A., Viaud, S., Zitvogel, L., Restifo, N. P., and Trinchieri, G. (2015) Microbiota modulation of myeloid cells in cancer therapy. Cancer immunology research 3, 103-109
6. Plottel, C. S., and Blaser, M. J. (2011) Microbiome and malignancy. Cell Host Microbe 10, 324-335
7. Dapito, D. H., Mencin, A., Gwak, G. Y., Pradere, J. P., Jang, M. K., Mederacke, I., Caviglia, J. M., Khiabanian, H., Adeyemi, A., Bataller, R., Lefkowitch, J. H., Bower, M., Friedman, R., Sartor, R. B., Rabadan, R., and Schwabe, R. F. (2012) Promotion of hepatocellular carcinoma by the intestinal microbiota and TLR4. Cancer Cell 21, 504-516
8. Goedert, J. J., Jones, G., Hua, X., Xu, X., Yu, G., Flores, R., Falk, R. T., Gail, M. H., Shi, J., Ravel, J., and Feigelson, H. S. (2015) Investigation of the association between the fecal microbiota and breast cancer in postmenopausal women: a population-based case-control pilot study. J Natl Cancer Inst 107
9. Tilg, H.; Adolph, T.E.; Gerner, R.R.; Moschen, A.R. The Intestinal Microbiota in Colorectal Cancer. Cancer Cell. 2018, 33, 954–964.
10. Miko, E.; Kovacs, T.; Sebo, E.; Toth, J.; Csonka, T.; Ujlaki, G.; Sipos, A.; Szabo, J.; Mehes, G.; Bai, P. Microbiome-Microbial Metabolome-Cancer Cell Interactions in Breast Cancer-Familiar, but Unexplored. Cells 2019, 8, 293.
11. Kovács, T.; Mikó, E.; Vida, A.; Sebő, É.; Toth, J.; Csonka, T.; Boratkó, A.; Ujlaki, G.; Lente, G.; Kovács, P.; Tóth D, Árkosy P, Kiss B, Méhes G, Goedert JJ, Bai P. Cadaverine, a Metabolite of the Microbiome, Reduces Breast Cancer Aggressiveness through Trace Amino Acid Receptors. Sci. Rep. 2019. Feb 4;9(1):1300
12. Miko, E.; Vida, A.; Kovacs, T.; Ujlaki, G.; Trencsenyi, G.; Marton, J.; Sari, Z.; Kovacs, P.; Boratko, A.; Hujber, Z.; Csonka T, Antal-Szalmás P, Watanabe M, Gombos I, Csoka B, Kiss B, Vígh L, Szabó J, Méhes G, Sebestyén A, Goedert JJ, Bai P. Lithocholic Acid, a Bacterial Metabolite Reduces Breast Cancer Cell Proliferation and Aggressiveness. Biochim. Biophys. Acta 2018, 1859, 958–974.
13. Yoshimoto, S.; Loo, T.M.; Atarashi, K.; Kanda, H.; Sato, S.; Oyadomari, S.; Iwakura, Y.; Oshima, K.; Morita, H.; Hattori, M.; et al. Obesity-Induced Gut Microbial Metabolite Promotes Liver Cancer through Senescence Secretome. Nature 2013, 499, 97–101.
14. Tan, J.; McKenzie, C.; Potamitis, M.; Thorburn, A.N.; Mackay, C.R.; Macia, L. The Role of Short-Chain Fatty Acids in Health and Disease. Adv. Immunol. 2014, 121, 91–119.
15. Luu, T.H.; Bard, J.M.; Carbonnelle, D.; Chaillou, C.; Huvelin, J.M.; Bobin-Dubigeon, C.; Nazih, H. Lithocholic Bile Acid Inhibits Lipogenesis and Induces Apoptosis in Breast Cancer Cells. Cell Oncol. (Dordr) 2018, 41, 13–24.
16. Kovács P, Csonka T, Kovács T, Sári Z, Ujlaki G, Sipos A, Karányi Z, Szeőcs D, Hegedűs C, Uray K, Jankó L, Kiss M, Kiss B, Laoui D, Virág L, Méhes G, Bai P, Mikó E. Lithocholic Acid, a Metabolite of the Microbiome, Increases Oxidative Stress in Breast Cancer. Cancers (Basel). 2019 Aug 27;11(9):1255.

Publications:

Microbial metabolite signaling in ovarian cancer: mechanistic links between the oncobiome and ovarian cancer
Sipos A, Ujlaki Gy, **Mikó E**, Maka E, Szabó J, Uray K, Krasznai Z, Bai P
Cancers (Basel) under review

Indolepropionic Acid, a Metabolite of the Microbiome, Has Cytostatic Properties in Breast Cancer by Activating AHR and PXR Receptors and Inducing Oxidative Stress.
Sári Z, **Mikó E**, Kovács T, Jankó L, Csonka T, Lente G, Sebő É, Tóth J, Tóth D, Árkosy P, Boratkó A, Ujlaki G, Török M, Kovács I, Szabó J, Kiss B, Méhes G, Goedert JJ, Bai P.Cancers (Basel). 2020 Aug 25;12(9):E2411. doi: 10.3390/cancers12092411.PMID: 32854297

Carbonic Anhydrase Inhibitor Acetazolamide Enhances CHOP Treatment Response and Stimulates Effector T-Cell Infiltration in A20/BalbC Murine B-Cell Lymphoma.
Méhes G, Matolay O, Beke L, Czenke M, Pórszász R, **Mikó E**, Bai P, Berényi E, Trencsényi G.Int J Mol Sci. 2020 Jul 15;21(14):5001. doi: 10.3390/ijms21145001.PMID: 32679833

Fecal expression of E. coli lysine decarboxylase (LdcC) is downregulated in E-cadherin negative lobular breast carcinoma
Sári Zs, Kovács T, Csonka T, Török M, Sebő É, Toth J, Tóth D, **Mikó E**, Kiss B, Szeőcs D, Uray K, Karányi Zs, Kovács I, Méhes G, Árkosy P, Bai P
Physiology International. 2020 May

Oncobiosis and Microbial Metabolite Signaling in Pancreatic Adenocarcinoma.
Kiss B, **Mikó E**, Sebő É, Toth J, Ujlaki G, Szabó J, Uray K, Bai P, Árkosy P.Cancers (Basel). 2020 Apr 25;12(5):1068. doi: 10.3390/cancers12051068.PMID: 32344895 Review.

The Microbiome as a Component of the Tumor Microenvironment.
Kovács T, **Mikó E**, Ujlaki G, Sári Z, Bai P.Adv Exp Med Biol. 2020;1225:137-153. doi: 10.1007/978-3-030-35727-6_10.PMID: 32030653 Review.

Nuclear Factor Erythroid 2-Related Factor 2 in Regulating Cancer Metabolism.
Smolková K, **Mikó E**, Kovács T, Leguina-Ruzzi A, Sipos A, Bai P.Antioxid Redox Signal. 2020 Mar 18. doi: 10.1089/ars.2020.8024. Online ahead of print.PMID: 31989830

Lithocholic Acid, a Metabolite of the Microbiome, Increases Oxidative Stress in Breast Cancer.
Kovács P, Csonka T, Kovács T, Sári Z, Ujlaki G, Sipos A, Karányi Z, Szeőcs D, Hegedűs C, Uray K, Jankó L, Kiss M, Kiss B, Laoui D, Virág L, Méhes G, Bai P, **Mikó E.**Cancers (Basel). 2019 Aug 27;11(9):1255. doi: 10.3390/cancers11091255.PMID: 31461945

Microbiome-Microbial Metabolome-Cancer Cell Interactions in Breast Cancer-Familiar, but Unexplored.
Mikó E, Kovács T, Sebő É, Tóth J, Csonka T, Ujlaki G, Sipos A, Szabó J, Méhes G, Bai P.Cells. 2019 Mar 29;8(4):293. doi: 10.3390/cells8040293.PMID: 30934972 Review.

Cadaverine, a metabolite of the microbiome, reduces breast cancer aggressiveness through trace amino acid receptors.
Kovács T, **Mikó E**, Vida A, Sebő É, Toth J, Csonka T, Boratkó A, Ujlaki G, Lente G, Kovács P, Tóth D, Árkosy P, Kiss B, Méhes G, Goedert JJ, Bai P.Sci Rep. 2019 Feb 4;9(1):1300. doi: 10.1038/s41598-018-37664-7.PMID: 30718646

Lithocholic acid, a bacterial metabolite reduces breast cancer cell proliferation and aggressiveness.
Mikó E, Vida A, Kovács T, Ujlaki G, Trencsényi G, Márton J, Sári Z, Kovács P, Boratkó A, Hujber Z, Csonka T, Antal-Szalmás P, Watanabe M, Gombos I, Csoka B, Kiss B, Vígh L, Szabó J, Méhes G, Sebestyén A, Goedert JJ, Bai P.Biochim Biophys Acta Bioenerg. 2018 Sep;1859(9):958-974. doi: 10.1016/j.bbabio.2018.04.002. Epub 2018 Apr 13.PMID: 29655782

Patents:

Bai P (29%), Goedert JJ (7%), Kovacs T (6%), **Mikó E** (29%), Vida A (29%) (2015) METHODS FOR DIAGNOSING BREAST CANCER. submitted for PCT (PCT/HU2018/050027)

Bai P (23%), Goedert JJ (4%), Kovacs T (23%), **Mikó E** (23%), Sebő É (2%), Tóth J (2%), Vida A (23%) Treatment and diagnosis of breast cancer. submitted for prelminary Hungarian patenting.

Poster presentations:

Mikó E, Kovács P, Kovács T, Csonka T, Ujlaki Gy, Sári Zs, Méhes G, Antal-Szalmás P, Watanabe M, Sebestyén A, Goedert J J, Bai P (2019) The role of lithocholic acid, a cytostatic bacterial metabolite in the pathogenesis of breast cancer. The way of microbiota in cancer conference, Milan

Kovács T, **Mikó E**, Vida A, Sebő É, Toth J, Csonka T, Boratkó A, Ujlaki Gy, Lente G, Kovács P, Tóth D, Árkosy P Kiss B, Méhes G, Goedert JJ, Bai P (2018) Cadaverine reduces breast cancer aggressiveness. Cell Symposia: Metabolites as Signalling Molecules, Seattle, USA

Mikó E, Kovács T, Ujlaki Gy, Kovács P, Csonka T, Hujber Z, Méhes G, Sebestyén A, Goedert JJ, Bai P (2018) Role of lithocholic acid in breast cancer pathology Cell Symposia: Metabolites as Signalling Molecules, Seattle, USA

Mikó E, Kovács T, Vida A, Csonka T, Sebő É, Tóth J, Sebestyén A, Méhes G, Goedert JJ, Bai P (2018) Cytostatic bacterial metabolites are reduced in early stage breast cancer - Bacterial metabolites in breast cancer initiation.Cell Symposia: Metabolites as Signalling Molecules, Seattle, USA

Mikó E, Vida A, Kovács T, Ujlaki Gy, Trencsényi Gy, Márton J, Sári Zs, Kovács P, Boratkó A, Hujber Z, Csonka T, Antal-Szalmás P, Watanabe M, Gombos I, Csoka B, Kiss B, Vígh L, Szabó J, Méhes G, Sebestyén A, Goedert JJ, Bai P (2018) Lithocholic acid, a bacterial metabolite modulates the behavior of breast cancer. EMBO Conference on the Microbiome, Heidelberg, Germany

Kovács T, **Mikó E**, Vida A, Sebő É, Toth J, Csonka T, Boratkó A, Ujlaki Gy, Lente G, Kovács P, Tóth D, Árkosy P, Kiss B, Méhes G, Goedert JJ, Bai P (2018) Cadaverine, a microbial diamine, reduces breast cancer aggressiveness. EMBO Conference on the Microbiome, Heidelberg, Germany

Mikó E, Vida A, Kovács T, Ujlaki Gy, Trencsényi Gy, Márton J, Sári Zs, Kovács P, Boratkó A, Hujber Z, Csonka T, Antal-Szalmás P, Watanabe M, Gombos I, Csoka B, Kiss B, Vígh L, Szabó J, Méhes G, Sebestyén A, Goedert JJ, Bai P (2018) Bioenergetic changes in breast cancer cells by lithocholic acid. European Bioenergetics Conference, Budapest, Hungary