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shown to be altered in cirrhosis. It should be noted that changes in microbial diversity and landscape occur not only in the feces of patients with cirrhosis, but also in other organs, tissues and biological fluids – liver, serum, saliva, ascitic fluid. It is interesting to find out if there is a relationship between urobiome and prognosis in cirrhosis.

Methods: 12 adult patients hospitalized with liver cirrhosis to the Gomel State Clinical Hospital N3 (Republic of Belarus) were included in the protocol of collection and low-temperature freezing of urine samples. After extraction and purification of DNA in each of the biological samples, PCR amplification of the V3-V4 region of the 16S rRNA gene was performed using modified universal bacterial primers (manufactured by Illumina, USA).

Results: The urine microbiota in cirrhotic patients who died within 30 days (n = 4) compared with patients who are alive (n = 8) is enriched with Fusobacteria taxa (8.57 × 10-4 and 1.85 × 10-5, p = 0.016) and Tenericutes (0.12 and 2.11 × 10-4, p = 0.048). The beta diversity of the urinary tract microbiota has differences in these groups of patients (p = 0.017).

Discussion/Conclusion: We have shown that the beta-biodiversity of the urine microbiome is significantly different in patients who are alive and died within 30 days.

21. Doxorubicin-induced arginine/citrulline cycle changes in rat model of non-alcoholic steatohepatitis

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Introduction: Doxorubicin (Dox) belongs to the group of anthracycline antibiotics, which are widely used in oncological diseases treatment. The high risk of toxic effects is the basis for studying of the main mechanisms of organs and systems Dox-induced injury development.

The aim – to investigate the peculiarities of Dox-induced arginine/citrulline cycle changes in rats with simulated non-alcoholic steatohepatitis (NASH).

Methods: The studies were conducted on 30 white non-linear adult rats, weighing 160–220 g. The rats were divided into 3 groups: I (n = 10) – rats (5 males, 5 females), on which NASH was modeled during 63 days, then from the 64th till 66th day they were injected with 1 ml of 0.9% sodium chloride solution intraperitoneally; II (n = 10) – rats (5 males, 5 females), on which NASH was modeled during 63 days, continued with Dox injections intraperitoneally 5 mg/kg/day from the 64th till the 66th day; III (n = 10) – rats (5 males, 5 females), which received a standard vivarium diet during 63 days, from the 64th to 66th day – 1 ml injections of 0.9% sodium chloride solution intraperitoneally. NASH modeling was hold using a diet containing a 42.8% fats mixture and a 4% fructose aqueous solution. On the 67th day, animals were euthanized under thiopental anesthesia at a dose of 50 mg/kg. A 10% liver homogenate was prepared, in which the concentration of arginine, citrulline, and arginase activity were determined.

Results: In the homogenate of NASH modeled group I rats, compared to the control, 1.4-fold increased arginine content was observed (0.39 3 0.02) vs. (0.27 3 0.02) Qmol/g; p = 0.003), with a simultaneous 3.2-fold decrease of arginase activity (0.80 3 0.12) vs. (2.61 3 0.21) Qmol/g/min; p = 0.002) and 1.7-fold decrease of citrulline content (32.57 3 2.26) vs. (55.67 3 1.23) Qmol/g; p < 0.0001).

In case of Dox administration in the rats of group II compared to group I, a 1.25-fold decrease of arginine was found in the liver homogenate (0.31 3 0.03) vs. (0.39 3 0.02) Qmol/g; p = 0.04), with a simultaneous 2-fold increase in arginase activity (1.62 3 0.23 vs. (0.80 3 0.12) Qmol/g/min; p = 0.009) and a 2.1-fold increase in citrulline content (68.54 3 3.37) vs. (32.57 3 2.26) Qmol/g; p = 0.002).

Discussion/Conclusion: NASH modeling is accompanied by an increased content of arginine in the liver homogenate, which can potentially be the reason for a decreased percentage of response to chemotherapy. Administration of doxorubicin to rats with NASH leads to an increase of citrulline concentration in the liver homogenate as an indirect marker of activation of the arginine conversion NO-synthase pathway.

22. Developing a preclinical mouse model to study image-guided stereotactic ablative radiotherapy and systemic treatment

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Introduction: Optimal treatment for Hepatocellular Carcinoma (HCC) is curative, either resection, liver transplantation or local ablation, however, only ~20% of patients are eligible for this. The majority of patients present with incurable disease, for whom transarterial chemoembolisation or systemic chemotherapy are palliative options. Unfortunately, both have low responses and high rates of recurrence and progression. Stereotactic Ablative Radiotherapy (SABR) is a newly approved treatment option for a subset of HCC patients who are not eligible for resection or other local treatments. Retrospective studies report that this treatment modality has both clinical efficacy and feasibility but prospective trials are required to better understand the role of SABR in HCC. SABR has the potential for good tumour control, but with recurrent and disseminated disease, there is a significant opportunity for integrating SABR into multimodal combination therapy. With this, there is a significant research opportunity in the preclinical space for models of SABR in HCC. Our aim is to develop a clinically relevant model to study targeted radiotherapy and systemic therapies in the treatment of HCC.

Methods: We have optimised an orthotopic transplant model injecting a mouse-derived HCC cell line into the immunocompetent murine liver. Using co-registered MRI/CT scans we have validated imaging-contrast agents to determine their suitability for CT-guided tumour identification. A timepoint optimization study examining 1) toxicity to the healthy liver, tumour and organs-at-risk and 2) comparing single, parallel opposed and arc beam strategies, was performed on a small animal radiation research platform delivering 20 Gy single fraction irradiation. Radiation-induced damage was assessed using immunohistochemistry and liver biochemistry.

Results: We have established a syngeneic orthotopic transplant model whereby transplantation of a murine-derived HCC cell line into the murine liver gives rise to an anatomically accurate mono-focal liver tumour in an immunocompetent setting. In keeping with clinical management and treatment planning, we have successfully implemented intravenous contrast enhanced imaging in our model to reliably detect and delineate liver tumours, enabling long-term longitudinal CT imaging follow-up. In the healthy liver, comparative levels of irradiation-induced DNA damage were observed between single, parallel opposed and arc beams. We demonstrate that 20 Gy single fraction irradiation, using an arc beam due to its clinical relevance, induces significant DNA-damage in tumours compared to non-irradiated tumour controls, with minimal off-target dosing to the liver and surrounding organs at risk. In addition, we report reduced proliferation (BrdU) and increased senescence (p21) in irradiated tumours. Finally, we observe, irradiation associated infiltration of CD8+ cells into the tumour 2 weeks after radiotherapy.