CDDF MULTI-STAKEHOLDER WORKSHOP

IMPACT OF THE MICROBIOME ON CANCER GROWTH AND THERAPY

Munich, Germany, 15 - 16 February 2019

The workshop on **Impact of the Microbiome on Cancer Growth and Therapy** was organised by the Cancer Drug Development Forum (CDDF) in cooperation with the Munich Tumour Centre, with the aim of enhancing the understanding of the human microbiome and its critical role in cancer growth and treatment.

The workshop consisted of two keynote lectures and sixteen 20-min lectures, including three from companies manufacturing products aiming to restore the human gut microbiome.

81 Participants attended the workshop. The majority was from academia (scientists, clinicians and students), ±30% was related to industry, and 10% had another occupation.

Abbreviations

AhR - aryl hydrocarbon receptor
BfArM - Bundesinstitut für Arzneimittel und Medizinprodukte
DDR - DNA damage response
FMT - faecal microbiota transfer
GvDH - graft versus host disease
IEC - intestinal epithelial cells

Gut microbiota, homeostasis and medication

The human microbiota consists of 10^{14} bacteria and other microbes; archaea, fungi, bacteriophages, viruses, protozoa and worms. The majority inhabits the intestine. Compiled data from microbiota cultures and sequencing of the microbiota genes (microbiome) identified that >90% of the >2,000 bacteria species belong to only a few bacterial phyla (Proteobacteria, Firmicutes, Actinobacteria and Bacteroidetes). Considerable variation in gut microbial phenotypes was found, host location showed the strongest associations with microbiota variations. Geographic variation effect sizes exceeded those of diseases, implying that gut-microbiota-based biomarkers for diseases may have only regional applications.

The intestinal epithelial cell (IEC) layer constitutes a rapidly self-renewing interface in intimate contact with the enteral environment and the immune system of the host, enabling intestinal homeostasis. Disturbances of this homeostasis via nutrition, microbiota, metabolism and inflammation can give rise

to altered microbiota phenotypes (intestinal dysbiosis) which are associated with the pathogenesis of both intestinal and extra-intestinal disorders such as autoimmune diseases, cancer, cardiovascular and metabolic diseases.

To maintain homeostasis immune cells such as CD4$^+$Foxp3$^+$ T regulatory cells and T helper 17 cells are required. The latter contribute to pathogen clearance at mucosal surfaces, but loss of these cells at mucosal surfaces has been linked to chronic inflammation and microbial translocation.

Mucosal immune responses are dominated by IgA, produced by plasma cells in the lamina propria. IgA is transported by IEC towards the outer mucus layer containing gut microbiota. Large numbers of eosinophils in the lamina propria are required for the development and maintenance of IgA plasma cells as eosinophil-deficient mice have few mucosal IgA+ plasma cells. Co-housing with wild-type mice increased the amount of IgA plasma cells and faecal IgA. It also induces IgA$^+$ germinal centres in Peyer's patches and TGF-β transcription in T follicular helper lymphocytes. TGF-β promotes T cell-independent IgA class switching. Further studies with faecal transplantation in mice showed that the presence of bacteriolytic mycoplasma Anaeroplasma in the gut microbiota from mice and human induced IgA through TGF-β class switching in T follicular helper lymphocytes in Peyer's patches.

Medication is responsible for 10% of the variation in the gut microbiome. In vitro studies on the interaction of 835 non-antibiotic drugs, at physiologically relevant concentrations, and of 38 human gut commensals showed that 78% of the antibacterial drugs, 24% of human-targeted drugs and 53% of anti-infective drugs have a broad impact on gut microbes. Calcium channel blockers, antimetabolites and antipsychotics are classes that consistently affect gut microbes.

The gut microbiome, chemo- and immunotherapy, and immune system

A paradigm shift is happening in cancer therapy from targeting tumour cells (chemotherapy, targeted agents) to targeting immune cells (immunotherapy). To improve cancer immunity, a better understanding is required of factors influencing the immune system such as the tumour environment and the human gut microbiome.

Studies in immunocompetent mice bearing subcutaneously transplanted syngeneic tumours show that disruption of the microbiota impaired the response to immune checkpoint blockers, cyclophosphamide and platinum compounds. Optimal responses to cancer therapy require an intact commensal gut microbiota that mediates its effects by modulating myeloid-derived cell functions in the tumour microenvironment.

Faecal microbiota transfer (FMT) from cancer patients who responded to immune checkpoint blockers into germ-free or antibiotic-treated mice ameliorated the antitumor effects of PD-1 blockade, whereas FMT from nonresponding patients failed to do so.

Clinical studies in the USA and Europe established that the composition of the gut microbiota modulates the effectiveness of anti-PD1 immunotherapy in melanoma, lung and renal cancer. The microbiota profile of responders and non-responders differed in the studies but also between the studies. Gut microbial species associated with positive response to anti- PD1 therapy are not co-ordinately expressed in different patients and the use of gut microbial profile as biomarker to predict a patients'anti-PD1 response profile is limited to local use as geographical and regional variation in gut microbiota dominates over host genetics.

Metabolomics profiling of gut microbiomes in mice showed that administration of various commensals including *Enterococcus hirea* modulates the immune system, the tumour microenvironment, ileum homeostasis, and promote antitumour cytotoxic T cell responses. *E. hirea* Th1-memory responses predict longer PFS. It is also an efficient anticancer probiotic with alkylating agents and immune checkpoint blockers in FMT- induced gut dysbiosis.

Metabolomics profiling of ilei, colons, livers and plasma after oral administration of various commensals, including *E. hirea*, show increased release of metabolites that can boost anticancer effects. Live but not pasteurized bacterium exert those effects. In addition, one bacterium strain is not the other – one enhance cyclophosphamide efficacy, the other not.

The gut microbiome, cellular and genotoxic stress in IEC

Dysbiosis reduced the thickness of the mucous layer on IEC and may disturb the protein-folding capacity of the endoplasmic reticulum (ER), thereby provoking a cellular state of "ER stress". One of the sensors of ER stress appears to be ATF6, an ER-spanning transmembrane protein. Upon ER stress, ATF6 translocates to the Golgi apparatus, where it is cleaved to liberate an active, soluble nuclear transcription factor to cause the transcription of ER chaperones. In newly developed transgenic mice expressing the activated form of ATF6 in intestinal epithelial cells, colonic tumorigenesis spontaneously developed and was dependent on the presence of the intestinal microbiota. Following Koch's postulates, the transfer of dysbiotic microbiota induced tumour formation in gnotobiotic mice. In conclusion, ATF6 was identified as a potential new oncogenic driver in 10% of CRC patients. ATF6 activation is conditioning the tumour niche and requires bacteria as a second hit.

Genome integrity of IEC is maintained by the DNA damage response (DDR) pathway which includes a set of DNA repair mechanisms, damage tolerance processes, and cell-cycle checkpoint pathways. Dependent upon the type and extent of the damage, the DDR causes either a transient cell-cycle arrest and DNA repair, senescence or elimination of damaged cells by apoptosis. Mouse data demonstrate that IL-22 is required for effective initiation of the DDR following DNA damage. IL-22 production is promoted by gut microbiota via IEC by group 3 innate lymphoid cells and γδ T cells.

Metabolites of glucosinolates, a group of phytochemicals contained in cruciferous vegetables, induce genotoxic stress in IEC. These metabolites are ligands of the aryl hydrocarbon receptor (AhR), and AhR-mediated signalling in group 3 innate lymphoid cells and γδ T cells controls the production of IL-22. Mice fed with diets depleted of glucosinolates produced only very low levels of IL-22 and, consequently, the DDR in epithelial cells of mice on a glucosinolate-free diet was impaired. This implies that IECs are able to protect their genome integrity by sensing of genotoxic compounds from the diet via AhR signalling in I group 3 innate lymphoid cells and γδ T cells and production of IL-22 on demand.

Gut commensals as immune-modulators in cancer

The gut microbiota play an important role in shaping the immune system. The immunological impact of only a few of these microbes have been elucidated e.g. segmented filamentous bacteria elicit a robust T helper 17 cells response and specific subsets of CD4+Foxp3+ regulatory T cells are induced by a range of individual or groups of microbes. To extend these studies, germ-free mice were monocolonised with each of 53 individual bacterial species, spanning the known human gut diversity,

and host immunologic adaptation to colonization was analysed. Most microbes had a distinct signature related to the number of types of immune cells that it affects. Unrelated bacterial strains from different phyla induce similar immune populations. The data suggest that microbial diversity in the gut ensures robustness of the microbiota's ability to generate a consistent immunomodulatory impact.

The gut microbiome as potential biomarker

Early detection of colorectal cancer reduces mortality, but better non-invasive screening assays are needed. Based on a limited study population, a classification model for the early detection of colorectal cancer was developed using metagenomic analysis of faecal samples. The accuracy of the model for colorectal cancer detection was similar to the standard faecal occult blood test and when both approaches were combined, the sensitivity improved >45% relative to the faecal occult blood test, while maintaining its specificity.

Meta-analysis of colorectal cancer metagenomic studies in Europe (4) and USA (1) showed that heterogeneity and confounding occurred. Most gut microbial species vary more by study than by disease and biological confounding (age, colonoscopy). The meta-analysis found a polymicrobial signature for colorectal cancer. A machine-learning approach showed that the models transfer well across training and test sets, but classifiers have an AUC between 0.6-0.8. An AUC value >0.8 is preferred for a robust outcome.

The gut microbiome and life span

Ageing of an organism is affected by extrinsic and intrinsic factors and the gut microbiome could be one of them. The killifish with a length of about 4 cm have a lifespan of 4 months is a good model system to investigate ageing. The fish show heart ageing (increase in lipofuscin and senescent cells), increased gut fibrosis and decreased bone density during its lifespan.

The gut bacterial diversity decreases with age in the killifish, their gut bacterial composition changed as well as function. Treatment of young fish with antibiotics increased their lifespan somewhat, but transfer of microbiota from young to middle-age fish extended the lifespan and delayed ageing behavioural. A role in the ageing process could be the ageing immune system. Thus, gut microbiota may play a key role in modulating vertebrate life span.

Gut microbiota and clinical microbial interventions

Cancer cachexia is a complex multi-organ syndrome characterised by body weight loss, weakness, muscle atrophy and fat depletion. The prevalence ranges 15-70%, depending on the tumour type. Limited therapeutic options are available and novel approaches to treat cachexia are needed.

Cachexia is driven by a variable combination of reduced food intake, metabolic changes, gut microbiota and inflammation. Mouse cancer cachexia models have an Intestinal dysbiosis (increased *Enterobacteriaceae*, decreased *Lactobacillus* spp.). Administration of a synbiotic, containing inulin-type fructans and live *Lactobacillus reuteri* 100-23, to leukaemic mice restored the Lactobacillus population, reduced the Enterobacteriaceae levels and also reduced hepatic cancer cell proliferation, muscle wasting and morbidity, and prolonged survival.

Further studies on host-microbiota interactions in cancer cachexia showed that the intestinal dysbiosis and gut barrier dysfunction are IL-6 driven, and *Klebsiella oxytoca*, one of the main Enterobacteriaceae, expands in cancer cachexia and acts as a gut pathobiont by altering gut barrier function in cachectic mice contributing to intestinal dysfunction.

To sort out whether these preclinical findings can be translated to cachectic patients, a clinical study (MicroAML) has been initiated to investigate the composition and activity of the gut microbiota of patients newly diagnosed for acute myeloid leukaemia, in relationship with their food habits and cachectic hallmarks. The recruitment for this study is currently ongoing in the academic hospitals in Leuven and Gent (Belgium).

Allogeneic stem cell transplantation is a curative treatment in leukaemia but can be a high-risk procedure. After high dose chemotherapy and total body irradiation to reduce the presence of leukaemic cells and inactivate the own immune cell system to reduce the chance of host versus graft rejection of the allogeneic stem cells, patients are vulnerable to bacterial translocation and infection, but also to graft versus host disease (GvHD). Gastrointestinal GvHD is the major cause of mortality. Infection and GvHD have been correlated to altered gut microbiome profiles. Loss of microbiota diversity early after stem cell transplantation is associated with worse overall survival.

The use of FMT as a microbial restorative intervention early after transplantation in allogeneic stem cells is being explored in early clinical studies. The results indicate that (third-party) FMT following allogeneic stem cell transplantation is feasible, safe, and associated with the expansion of recipient microbiome diversity. Prospective trials are ongoing and planned to further investigate the effect of FMT in allogeneic stem cell transplantation.

The gut microbiota and obesity

Indoleamine 2,3-dioxygenase (IDO) is an enzyme mainly expressed in antigen presenting cells (macrophages, dendritic cells) and intestinal epithelial cells in response to inflammatory stimuli, and it catalyses the degradation of tryptophan along the kynurenine pathway. IDO has immunosuppressive effects, is important in host defence against microbial infection and involved in various inflammatory diseases such as atherosclerosis, and cardiovascular diseases.

Studies in mice show that obesity is also associated with increased IDO activity in the intestine, accompanied by a shift of tryptophan metabolism and IL-22 production toward kynurenine production. Inhibition or absence of IDO protects against intestinal dysbiosis in the obesity context. A similar shift in tryptophan metabolism was found in data from non-obese, obese and diabetic patients.

Intratumoural bacterial flora (oncobiome) and efficacy of chemotherapeutics

Bacteria are present in human tumours and also in tumours in immunocompetent mice. Certain bacteria species are causative for malignancies. Clinical and preclinical studies show that, in many cases, intratumoural bacteria are the result of spontaneous infection of established tumours. Tumour specific factors such as leaky vasculature, low oxygen tension, nutrients and immune suppression support bacterial growth whereas growth is not supported in healthy organs. Microbiome sequencing shows that many/all patients have many bacteria in their tumours and even in non-malignant tissue contain some bacteria. The microbiota profile of cancer patients differs from healthy individuals.

5

In vitro and in vivo studies with bacteria and tumour cell lines demonstrated that local bacteria can either inhibit, improve and not affect the efficacy of chemotherapeutics. The ability of bacteria to colonise different parts of the human body could be exploited in the future for intratumoural production of therapeutics and/or diagnostic purposes.

Regulatory aspects of microbial interventions

The favourable results of FMT in the treatment of patients with *Clostridium difficile* infection created a need for regulatory guidance on FMT use and manufacturing in Germany and Europe.

The EU has no harmonised definition or regulations on FMT. FMT is in Europe under control of the respective national drug regulatory agencies. BfArM is the German national competent authority responsible for microbiota therapeutics. It distinguishes minimally (FMT) and (highly) manipulated microbiota products (a defined composition of stool-derived bacterial strain(s) produced under a controlled manufacturing process). BfArM considers FMT as a medicinal product that does not require a manufacturing authorisation when it is manufactured under the responsibility of a physician for personal use for a specific patient. However, the treating physician bears complete responsibility and (health) insurance companies will not provide financial compensation. FMT for individual treatment is exempt from the need for marketing authorisation when it is manufactured from substances of human origin and the manufacturing is carried out in a pharmacy. In this respect, a Good Manufacturing Practice facility in Germany would be desirable but is probably too expensive in an academic setting.

A clinical trial application is required if clinical trials are to be performed as well as a manufacturing authorisation. For advice on FMT or highly manipulated microbiota products consult the scientific advice by EMA or competent national authorities during the development of the product. Small research facilities/start-ups can contact the innovation office.

Microbial products as intervention

Three companies (2 USA, 1 France) presented their approach, pipeline, disease applications and products. The products varied between a product from the pooled stool of healthy donors (FMT), a selected single bacterium strain (monoclonal microbial) to defined consortia of bacterial strains to restore the gut microbiome from patients. The composition varies depending on the intended application (cancer immunotherapy, food allergy, inflammatory bowel disease, *Clostridium difficile*, etc.). Most products in the pipeline are yet in preclinical/manufacturing development and a couple will start clinical phase I studies in 2019/2020.

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